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New Drugs

Cannabinoids: A new hope for breast cancer therapy?

María M. Caffarel [1], Clara Andradas, Eduardo Pérez-Gómez, Manuel Guzmán, Cristina Sánchez *

Dept. Biochemistry and Molecular Biology I, School of Biology, Complutense University-CIBERNED-IRYCIS, Madrid, Spain

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ABSTRACT

Breast cancer is a very common disease that affects approximately 1 in 10 women at some point in their lives. Importantly, breast cancer cannot be considered a single disease as it is characterized by distinct pathological and molecular subtypes that are treated with different therapies and have diverse clinical outcomes. Although some highly successful treatments have been developed, certain breast tumors are resistant to conventional therapies and a considerable number of them relapse. Therefore, new strategies are urgently needed, and the challenge for the future will most likely be the development of individualized therapies that specifically target each patient's tumor. Experimental evidence accumulated during the last decade supports that cannabinoids, the active components of *Cannabis sativa* and their derivatives, possess anticancer activity. Thus, these compounds exert anti-proliferative, pro-apoptotic, anti-migratory and anti-invasive actions in a wide spectrum of cancer cells in culture. Moreover, tumor growth, angiogenesis and metastasis are hampered by cannabinoids in xenograft-based and genetically-engineered mouse models of cancer. This review summarizes our current knowledge on the antitumor potential of cannabinoids in breast cancer, which suggests that cannabinoid-based medicines may be useful for the treatment of most breast tumor subtypes.

Introduction

Cannabinoids are a family of unique compounds synthesized by *Cannabis sativa* (marijuana) that have not been found as yet in any other plant. The main representative cannabinoid, owing to its high abundance in the plant and its strong biological activity, is Δ^9-tetrahydrocannabinol (THC).[1] For decades, and mainly due to their lipophilic nature, it was assumed that cannabinoids exerted their effects by perturbing the biophysical properties of biological membranes.[2] This scenario changed dramatically in 1990, when the first cannabinoid-specific membrane receptor (CB_1) was characterized and cloned.[3] This discovery boosted the scientific research on cannabinoids, which currently constitutes a very active field in biomedicine. The term cannabinoid includes now not only the marijuana-derived compounds that are structurally related to THC (phytocannabinoids), but also the synthetic molecules that activate the same primary targets as THC (synthetic cannabinoids) and a family of endogenously produced compounds that engage cannabinoid receptors (endocannabinoids), of which anandamide (arachidonoylethanolamide, AEA)[4] and 2-arachidonoylglicerol (2-AG)[5,6] are the main – if not only – representatives. Two G protein-coupled receptors (GPCRs) that are selectively engaged by cannabinoids have been cloned so far (CB_1 and CB_2),[3,7] and some evidence indicates that cannabinoids may act by binding to additional receptors such as the vanilloid receptor 1 (TRPV1) and the orphan GPCR GPR55.[8] The endocannabinoids, together with their receptors and the proteins in charge of their transport, synthesis and degradation, constitute the so called endocannabinoid system, which is involved in the control of a wide variety of biological functions such as motor behavior, memory and learning, pain, the immune response or bone physiology, just to mention a few.[2]

Cannabinoids and cancer

The therapeutic properties of marijuana have been known for millennia, but the use in the clinic of either plant-derived preparations or pure cannabinoids is still very limited. To date, only three cannabinoid-based medicines can be prescribed, and for very specific indications. The orexigenic and anti-cachexic properties of cannabinoids are exploited by Marinol® (oral capsules of dronabinol – synthetically generated THC) to manage weight loss associated to wasting syndrome in patients with AIDS. Sativex® (nabiximols, an oromucosal spray containing plant extracts enriched in THC and cannabidiol at an approximate 1:1 ratio) has been recently approved in several European countries, Canada and New Zealand for the relief of spasticity associated to multiple sclerosis, and in Canada for the treatment of neuropathic pain in the same disease. In the context of cancer, it is well established that cannabinoids have

* Corresponding author. Address: Dept. Biochemistry and Molecular Biology I, School of Biology, Complutense University, C/ José Antonio Novais, 2, 28040 Madrid, Spain. Tel.: +34 913944668; fax: +34 913944672.
E-mail address: cristina.sanchez@quim.ucm.es (C. Sánchez).
[1] Present address: Dept. Pathology, University of Cambridge, Cambridge, UK.

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antiemetic properties,[9] and, in fact, Marinol® and Cesamet® (oral capsules of nabilone – a synthetic THC analog) can be prescribed to prevent nausea and vomiting elicited by standard chemotherapeutic regimes. In addition, Sativex® is approved in Canada for the treatment of cancer-associated pain.

Aside from these palliative actions, recent preclinical evidence suggests that cancer patients might benefit from cannabinoids in an additional manner: since the late 1990s, an important amount of experimental data has shown that these compounds exert anti-tumor effects in different models of cancer, ranging from cell cultures to xenografted and genetically engineered mice.[10] Cannabinoid anti-tumor action relies on the blockade of several hallmarks of tumor progression. Thus, they inhibit uncontrolled cancer cell growth (by inhibiting cancer cell proliferation and by inducing cancer cell death by apoptosis) and impair tumor angiogenesis and metastasis. Interestingly, some – if not all – of these effects have been observed in tumor cells from very different origin, including gliomas, melanomas, carcinomas of the breast, skin, lungs, liver, pancreas, colon, prostate, and lymphomas amongst others, which indicates that cannabinoid anti-tumor action has a general rather than tumor-type specific nature.[10]

Anti-tumor effects of cannabinoids in breast cancer

Breast cancer is the most common malignant disease among Western women. Although the rates of mortality have declined since the late 1990s, mainly due to adjuvant systemic therapy and earlier detection by palpation and mammograms, certain breast tumors remain resistant to conventional therapies. In addition, current treatments have side effects that substantially affect the patients' quality of life.[11–13] It is therefore obvious that new therapeutic strategies are needed for the management of this condition. As breast cancer is an extremely heterogeneous disease that comprises tumors that are very diverse in terms of molecular portraits, prognosis and treatments,[13] in this review we will distinguish the three main breast cancer subtypes according to classical molecular profiles: hormone receptor-positive, HER2-positive and triple-negative tumors. Although the strength of the experimental data is different in each case, evidence obtained so far suggests that cannabinoid-based medicines may be useful for the treatment of these three breast cancer subtypes.

Cannabinoids and hormone-sensitive breast cancer

The presence of estrogen receptors (ER) and/or progesterone receptors (PR) in breast cancer cells, as determined by immunohistochemistry-based methods, defines a subgroup of breast tumors that may respond to endocrine therapy. Specifically, these patients are treated with surgical and/or pharmacological approaches that block estrogenic signaling, which has pro-proliferative and pro-survival features. The targeted strategies include the removal of the endogenous source of estrogens (the ovaries) and/or the use of selective estrogen receptor modulators, such as the widely used tamoxifen, or inhibitors of aromatase, the main enzyme responsible for estrogen synthesis.[14]

It has been demonstrated that cannabinoids modulate pivotal tumor progression-related aspects of ER^+/PR^+ breast cancer cells. Thus, anandamide inhibits basal[15,16] and prolactin- and nerve growth factor (NGF)-induced proliferation[15,17] of MCF-7 and EFM-19 cells in culture. This effect is mediated by the activation of CB_1 receptors[15–17] and is not accompanied by cancer cell death.[15] Anandamide produces this anti-proliferative action by blocking the progression through the cell cycle, specifically by preventing the transition from the G1 to the S phase,[15] and by inhibiting adenylyl cyclase and thus activating the Raf-1/ERK/MAPK cascade, which, upon sustained activation, ultimately down-regulates prolactin and TrkA NGF receptors[15–17] (Fig. 1).

The proliferation of the ER^-/PR^+ human breast cancer cell line EVSA-T is also decreased in response to THC.[18,19] Once again, the cell cycle is targeted: a blockade in the transition from G2 to mitosis via CB_2 receptors, produced by the inhibition of CDK1, was observed.[19] Cell cycle arrest is ensued by apoptotic cell death,[18,19] and the activation of the transcription factor JunD, owing to upregulation of gene expression and translocation to the nucleus, is essential for these actions[18] (Fig. 1).

Besides cancer cell proliferation, cannabinoids impair ER^+/PR^+ cancer cell migration and invasion in culture. Specifically, selective activation of CB_2 receptors in MCF-7 cells that overexpress the chemokine receptor CXCR4 inhibited chemotaxis and wound healing as induced by the CXCR4 ligand CXCL12[20] (Fig. 1). The CXCL12/CXCR4 signaling axis plays a pivotal role in directing breast cancer cells to distant sites[21] and, therefore, the aforementioned finding suggests that cannabinoids may modulate hormone-sensitive breast cancer metastasis. However, the experimental support for this notion is still weak and further research with more complex models should be performed to validate it.

Cannabinoids and HER2-positive breast cancer

The breast tumors that express the tyrosine kinase receptor HER2, as determined by immunohistochemistry and fluorescence *in situ* hybridization (FISH) approaches, constitute another well defined breast cancer histopathological subtype. HER2 belongs to the epidermal growth factor receptor (EGFR) family, which consists of four members (ErbB1/HER1/EGFR, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4). They all have intrinsic tyrosine kinase activity and, upon ligand binding and subsequent dimerization, they activate a number of oncogenic processes, including cell proliferation and survival.[22] The outcome of these patients has considerably improved since the incorporation to the clinics of Herceptin® (trastuzumab, a humanized monoclonal antibody against the extracellular domain of HER2). Other compounds are in use or under development to overcome trastuzumab resistance, the most prominent being Tykerb® (lapatinib), a dual EGFR/HER2 tyrosine kinase inhibitor.[13]

Strong preclinical evidence suggests that cannabinoids may be useful for the treatment of this particular subset of patients. Thus, THC produces a significant anti-tumor action in MMTV-neu mice,[23] a well established and clinically relevant model of HER2-positive metastatic breast cancer.[24] THC treatment reduces not only tumor growth but also the number of tumors generated per animal and the percentage of animals with lung metastases.[23] THC action relies on the impairment of cancer cell proliferation, via inhibition of the pro-tumorigenic kinase AKT, and on the induction of cancer cell death by apoptosis.[23] A reduction in the number of tumor blood vessels is also observed, suggesting that tumor angiogenesis is impaired by THC[23] (Fig. 2).

Xenograft-based approaches strengthen the hypothesis that HER2-overexpressing tumors may be sensitive to cannabinoids. Two different cell lines isolated from MMTV-neu-derived tumors were injected either subcutaneously in immune-deficient mice[23] or orthotopically in immune-competent syngenic FVB mice[20] and treated with THC[23] and/or CB_2-selective agonists.[20,23] In both cases, a significant reduction in tumor growth was observed, mediated by the inhibition of AKT in one case[23] and accompanied by a decrease in the levels of activated ERK and CXCR4 in the other[20] (Fig. 2).

Of interest, the anti-tumor effect of cannabinoids in all the HER2-positive breast cancer models used so far is mediated by the activation of CB_2 receptors (Fig. 2). Thus, the anti-tumor action of THC in the MMTV-neu model is mimicked by the CB_2-selective agonist JWH-133.[23] In the same line, the growth-inhibiting effect

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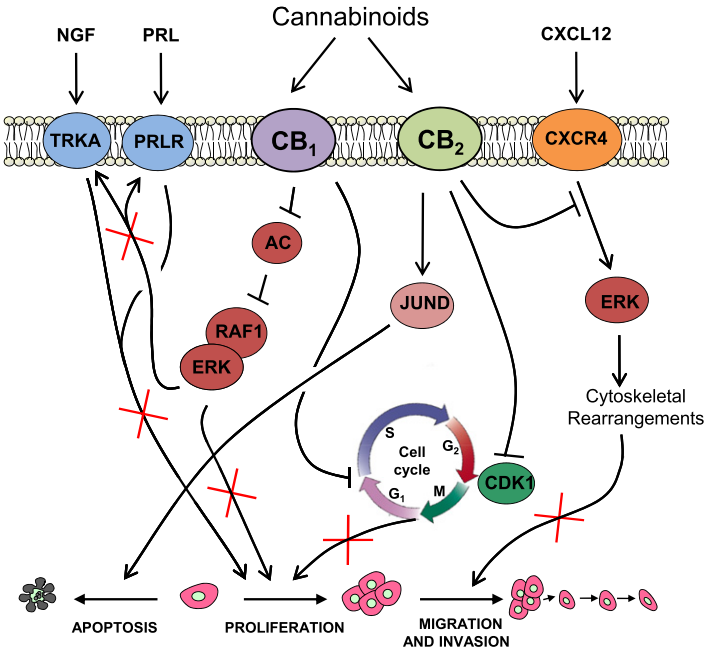

Fig. 1. Mechanism of cannabinoid-receptor mediated anti-tumor action in hormone-sensitive breast cancer cells. Cannabinoids, by binding CB_1 and/or CB_2 receptors, inhibit ER^+ and/or PR^+ breast cancer cell proliferation in culture by blocking the progression through the cell cycle at different levels. Likewise, they hamper both basal and prolactin (PRL)- and nerve growth factor (NGF)-induced proliferation *in vitro* (by downregulating NGF and PRL receptors, respectively). Moreover, binding of cannabinoids to CB_2 receptors induces cancer cell death by apoptosis, mediated by the activation of the transcription factor JUND, and inhibits chemokine-induced cancer cell migration and invasion in culture, by blocking ERK-induced cytoskeletal rearrangements. TRKA, high-affinity NGF tyrosine kinase receptor A; PRLR, prolactin receptor; AC, adenylyl cyclase; CDK1, cyclin-dependent kinase 1; CXCR4, chemokine (C-X-C motif) receptor 4; CXCL12, chemokine (C-X-C motif) ligand 12.

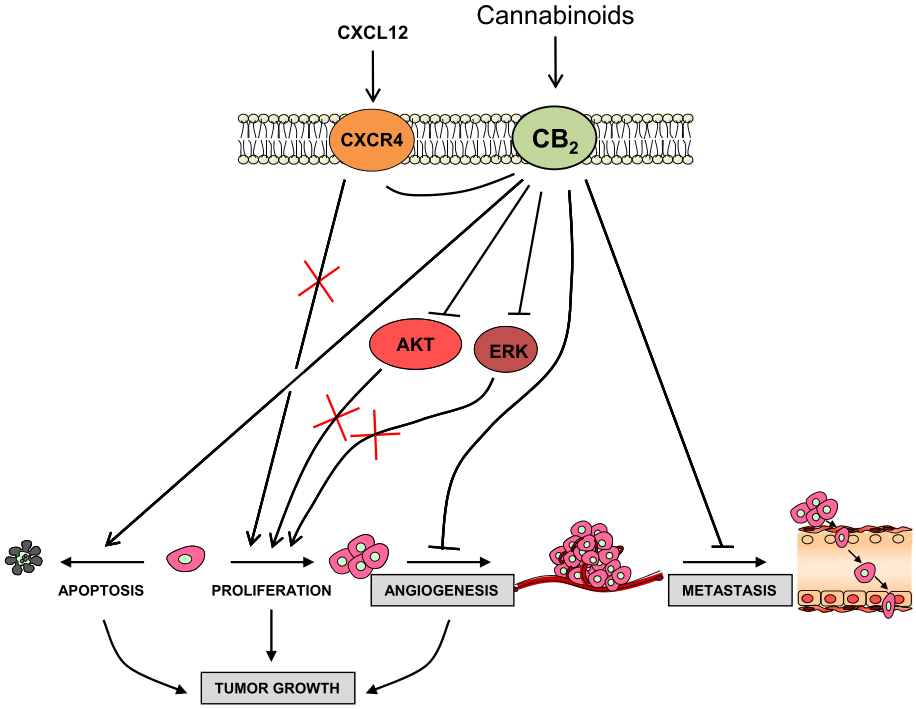

Fig. 2. Mechanism of cannabinoid-receptor mediated anti-tumor action in HER2-positive breast cancer. Cannabinoids, by activating CB_2 receptors in HER2-overexpressing breast cancer cells, block different hallmarks of cancer both *in vitro* and in mice *in vivo* (squared grey boxes). They block cancer cell proliferation in culture and in tumors by inhibiting the protumorigenic kinases AKT and ERK, and by reducing the levels of the activated form of the chemokine (C-X-C motif) receptor 4 (CXCR4). In addition, cannabinoids induce apoptosis of cancer cells *in vitro* and *in vivo*, and inhibit tumor angiogenesis. All these events converge in the inhibition of tumor growth in animal models of HER2-positive breast cancer. Additionally, the generation of lung metastases is also impaired by activation of CB_2 receptors *in vivo*.

on orthotopic xenografts is produced by another CB_2-selective agonist (JWH-015)[20] and the effect on subcutaneous xenografts is mimicked by JWH-133 and prevented by the CB_2-selective antagonist SR144528.[23] The involvement of this particular receptor in

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cannabinoid anti-tumor action may have important clinical implications since the psychotropic effects associated to these compounds are mediated by the activation of CB_1 (and not CB_2) receptors present in the brain.[25] Therefore, a CB_2-directed therapy may be, in principle, efficacious in curbing the growth of these tumors and would be devoid of the classical cannabis-associated psychotropic side effects.

Cannabinoids and triple-negative breast cancer

The remaining group of breast tumors according to immunopathological criteria is the triple-negative one. This definition refers to the lack of expression of ER, PR and HER2. To date, there is no standard targeted therapy for these patients, whose prognosis is very poor as a group.[12] Efforts are being made to improve chemotherapy responses and they include, for example, the combined use of angiogenesis inhibitors such as Avastin® (bevacizumab) and poly (adenosine diphosphate–ribose) polymerase (PARP) inhibitors.[12,13]

Both *in vitro* and *in vivo* preclinical evidence indicates that triple-negative breast cancer may be treated with cannabinoids. A battery of synthetic cannabinoids has been tested in the human triple-negative breast cancer cell line MDA-MB-231, and all of them produced an inhibition of cell proliferation.[19,26–31] For example, the metabolically stable analog of anandamide Met-F-AEA reduces MDA-MB-231 proliferation without inducing cell death[32] by arresting cells in the S phase of the cell cycle.[26] This effect is accompanied by a decrease in the activity of the cyclin-dependent kinase CDK2 and the modulation of the levels of other important cell cycle regulators.[26] CB_1 receptors seem to be the primary target of Met-F-AEA action in this model[26,32] (Fig. 3). The synthetic cannabinoids WIN 55,212-2 (a CB_1 and CB_2 mixed agonist) and JWH-133 also produce an inhibition of MDA-MB-231 proliferation by blocking the progression trough the cell cycle, but in this case (i) the cells are retained in the G0/G1 compartment, (ii) this effect is connected to apoptosis, and (iii) it is mediated by both CB_1 and CB_2 and receptors[30] (Fig. 3). Whether these differences are agonist-specific or due to other experimental issues has not been clarified.

Interestingly, the cannabinoid proliferation-inhibiting effect is reproduced *in vivo*, both in a xenograft-based and in the PyMT genetically engineered model of triple-negative breast cancer.[30] In these two models, a significant reduction in tumor growth is observed upon JWH-133 treatment, and the analysis of the tumors revealed a significant decrease in the number of cancer cells undergoing proliferation.[30] Of note, decreased immunoreactivity of the vascular marker CD31 was found in cannabinoid-treated tumors, which suggests that tumor angiogenesis is also hampered by JWH-133.[30] In line with the idea that cannabinoids impact not only proliferation but also other tumor progression-related features of triple-negative cells, it was found that migration of MDA-MB-231 cells in culture is blocked by Met-F-AEA, WIN 55,212-2, JWH-133 and JWH-015.[20,30,32,33] As for the effects on proliferation, Met-AEA action on migration is mediated by CB_1 receptors,[32,33] while that of WIN 55,212-2 and the JWH compounds is mainly produced by activation of CB_2 receptors[20,30] (Fig. 3). Engagement of CB_1 receptors by Met-F-AEA leads to the inhibition of the focal adhesion kinase (FAK)/Src[32] and RhoA-ROCK pathways,[33] while the inhibitory effect produced by WIN 55,212-2 or JWH-133 via CB_2 receptors is accompanied by the inhibition of the COX-2/PGE_2 axis[30] (Fig. 3). Both signaling systems play important roles in driving cell migration and metastasis.[34–36] Finally, JWH-015 reduces CXCL12-induced cell migration and invasion of a highly metastatic MDA-MB-231-derived cell line by inhibiting ERK and cytoskeletal focal adhesion and stress fiber formation via CB_2 receptors[20] (Fig. 3), the latter being crucial events in cancer invasion and metastasis.[37]

Together, these observations suggest that cannabinoids, via CB_1 and/or CB_2 receptors, confer a less invasive phenotype to triple-negative breast cancer cells in culture, and allows hypothesizing that these compounds may reduce the cancer cell metastatic potential *in vivo*. Importantly, this hypothesis has been confirmed in an animal model of lung metastasis. Thus, WIN 55,212-2 and JWH-133 reduces the number of lung metastases generated by injection of MDA-MB-231 cells into the lateral tail vein, an effect that is completely prevented by the combined pharmacological blockade of CB_1 and CB_2 receptors[30] (Fig. 3).

Phytocannabinoids other than THC have been shown as well to exert anti-tumor actions in breast cancer. The most studied in the triple-negative context has been cannabidiol (CBD). This compound displays low affinity for CB_1 and CB_2 receptors[8] and, although its mechanism of action is not completely understood, it is emerging as an attractive potential therapeutic tool for a number of conditions.[38,39] Several groups have reported that CBD reduces MDA-MB-231 cell proliferation in culture, but conflicting results were obtained as regards the molecular bases underlying this effect, especially concerning cannabinoid receptor involvement. Ligresti and coworkers observed an induction of apoptosis that was partially prevented by CB_2 and TRPV1 antagonists.[27] Based on these results and previous data from the same group, the authors proposed that CBD action is produced by the combination of the direct activation of TRPV1 receptors by CBD, the indirect activation of CB_2 receptors by anandamide (whose deactivation is inhibited by CBD)[40] and the activation of other uncharacterized and unique targets of CBD.[27] On the other hand, a recent paper rules out the involvement of CB_1, CB_2 and TRPV1 receptors in CBD-induced apoptosis of MDA-MB-231 cells.[31] In this case, CBD induces endoplasmic reticulum (ER) stress and a subsequent inhibition of the AKT/mTORC1 axis that leads to autophagy and mitochondria-driven apoptosis.[31] The induction of ER stress and/or autophagy followed by apoptosis has been previously associated to cannabinoid anti-tumor action in different types of cells, including glioma,[41] pancreatic cancer,[41,42] hepatocellular carcinoma,[43] rhabdomyosarcoma[44] and mantle cell lymphoma,[45] which suggests that this may be a general mechanism of cancer cell death induced by cannabinoids.[10]

Within the complex and controversial mechanistic explanations for CBD action in cancer cells, the increase in reactive oxygen species (ROS) seems to be the most accepted and reproducible. Although it is known that CBD *per se* is a potent antioxidant,[38] it has been observed that it triggers a signaling mechanism that involves the generation of ROS in glioma,[46] leukemia[47] and MDA-MB-231 breast cancer cells.[27–29,31] The molecular explanation for this apparent divergence has not been elucidated yet.

The inhibition of breast cancer cell proliferation by CBD has been corroborated *in vivo*. Treatment of subcutaneous xenografts generated from MDA-MB-231 cells in immune-deficient mice resulted in a marked reduction of tumor growth.[27] Similar results were observed in orthotopic xenografts generated from 4T1 triple-negative breast cancer murine cells in syngenic BALB/c mice, although in this case tumors acquired resistance to CBD after 3 weeks of treatment and, from that time onwards, grew as fast as the vehicle-treated tumors at the end of the experiment.[29]

As for other cannabinoids, CBD action on triple-negative breast cancer cells impacts not only proliferation but also metastasis-related capabilities. Thus, MDA-MB-231 and 4T1 cell invasion was hampered in culture by CBD.[28,29] Most important, this compound reduced the number of lung metastases generated by intraplantar injection of MDA-MB-231 cells,[31] by injection of 4T1 cells into the tail vein, and by spontaneous formation from 4T1 orthotopic xenografts.[29] The down-regulation of Id1 (an inhibitor of basic helix-loop-helix transcription factors) played a pivotal mechanistic role in these CBD anti-tumor actions.[28,29]

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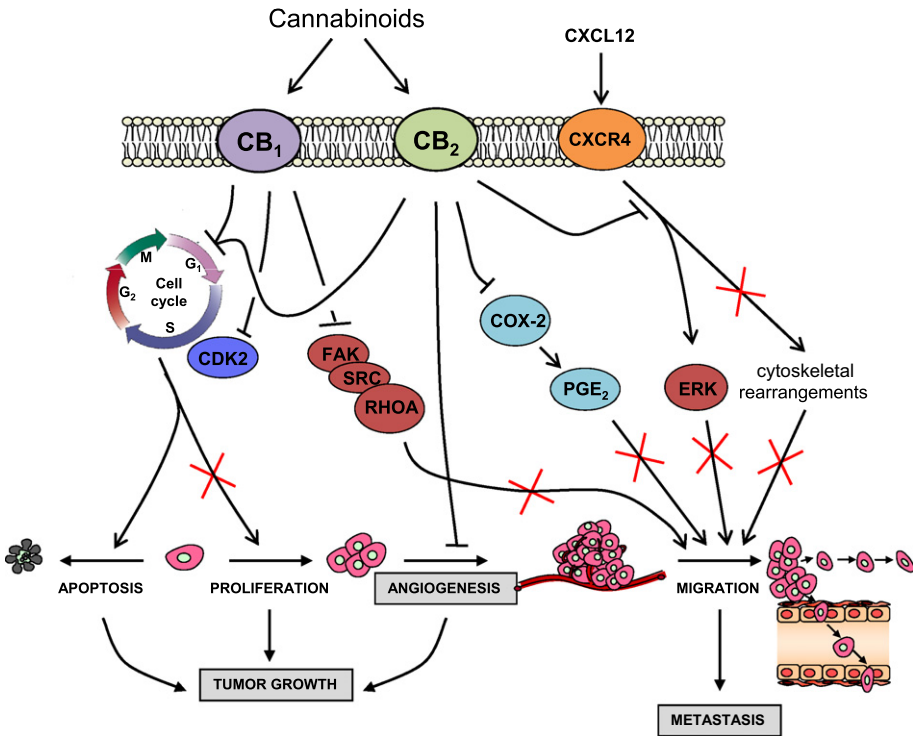

Fig. 3. Mechanism of cannabinoid-receptor mediated anti-tumor action in triple-negative breast cancer. Activation of CB_1 and/or CB_2 receptors inhibit tumor progression at different steps both *in vitro* and in mice *in vivo* (squared grey boxes). Engagement of CB_1 receptors by cannabinoids blocks cell cycle progression by arresting cells in G1 or by preventing their exit from the S phase. CB_1 activation also hampers the FAK/SRC/RHOA pathway, resulting in an inhibition of cell migration in culture. Cell migration blockade is also achieved by CB_2 activation through the inhibition of COX-2 and ERK and the blockade of chemokine-induced cytoskeletal rearrangements. In addition, engagement of CB_2 receptors inhibits angiogenesis *in vivo*. Together, these actions produce an inhibition of tumor growth and metastasis in animal models of triple-negative breast cancer. CDK2, cyclin-dependent kinase 2; FAK, focal adhesion kinase; COX-2, cycloxigenase-2; PGE2, prostaglandin E2; CXCR4, chemokine (CX- C motif) receptor 4; CXCL12, chemokine (C-X-C motif) ligand 12.

Pro-tumor effects of cannabinoids in breast cancer

Although the vast majority of reports published so far show that cannabinoids induce anti-tumor responses not only in breast but in many other types of cancer, a reduced number of articles have reported pro-tumor actions in response to cannabinoids. Thus, McKallip and coworkers found that THC, via CB_2 receptors, enhanced the growth of 4T1-derived xenografts and metastases by the inhibition of the anti-tumor immune response.[48] Similarly, the growth of xenografted lung tumors was augmented by THC via CB_2 receptors[49] and by methanandamide via a receptor-independent mechanism.[50] However, other reports demonstrate an important contribution of the immune system in cannabinoid-induced anti-tumor action. For example, the growth of melanoma xenografts was inhibited more efficiently by cannabinoids in immune-competent mice than in immune-deficient mice.[51] In addition, a prolonged treatment of immune-competent rats with THC decreased the incidence of tumors and enhanced overall animal survival.[52] Additional studies should be performed to clarify whether cannabinoids activate or inhibit the immune surveillance of tumors.

Takeda and coworkers reported that THC enhances the proliferation of MCF-7 cells in culture.[53,54] Interestingly, both in this case as in the work by McKallip and coworkers,[48] the cells in which pro-tumor effects of cannabinoids were observed expressed undetectable/very low levels of cannabinoid receptors. It would be important to confirm this observation in other breast cancer cell lines to determine whether the lack of CB_1/CB_2 receptors could be a marker of resistance to cannabinoid anti-tumor action.

Additionally, the pro-tumor effect of cannabinoids in certain conditions could be explained by the biphasic effects that these compounds have in multiple processes such as the control of appetite or anxiety.[55] Indeed, we have observed a biphasic effect of cannabinoids in the proliferation of various cancer cell lines: while low doses of cannabinoids promote cell proliferation (unpublished observations), higher doses exert the well described anti-proliferative effect. However, only two works have reported so far pro-tumor effects of cannabinoids *in vivo*.[48,50]

Expression of cannabinoid receptors in human breast tumors

There is compelling evidence demonstrating the expression of cannabinoid receptors in human breast cancer biopsies.[19,23,30] Of interest, a correlation between CB_2 receptor expression and tumor aggressiveness has been found.[19] Thus, the levels of CB_2 mRNA were higher in ER^-/PR^- tumors than in ER^+/PR^- tumors, in HER2-positive tumors than in HER2-negative tumors, and in high histological grade than in low histological grade tumors.[19] This association between cannabinoid receptor expression and tumor aggressiveness has been observed as well in other types of tumors such as gliomas,[56–58] prostate[59] and colorectal cancers,[60] which may indicate that the endocannabinoid system is up-regulated in cancer. It is not clear yet whether this up-regulation is protective or pro-tumorigenic, and this question certainly deserves further investigation. To address this issue, it would be interesting to analyze, for example, the levels of endocannabinoids as well as of their metabolizing enzymes in breast tumors, and whether the lack of cannabinoid receptors in mice reduces or enhances breast tumor generation and/or progression. Conflicting reports have been published in other types of cancer, showing for example that the lack of cannabinoid receptors in mice protects from skin carcinogenesis[61] but accelerates intestinal cancer growth.[62]

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Of interest, the sensitivity of human breast cancer cells to cannabinoids in culture correlates with their aggressiveness. For example, we showed that ER^- cell lines were more susceptible to cannabinoid treatment than ER^+ cells.[19] In addition, Grimaldi et al. observed that Met-F-AEA inhibited the proliferation of the highly metastatic MDA-MB-231 cell line more efficiently than that of the poorly invasive and non-metastatic T47D cell line.[32]

Other potential benefits of cannabinoids for the treatment of breast cancer

A general feature of cannabinoid anti-tumor action in breast and other types of tumors is the lack of toxicity on non-tumor cells. Thus, the inhibition of cancer cell proliferation upon cannabinoid treatment was not evident in non-transformed human mammary epithelial cells.[18,19,27,31] This observation, that has been made as well in other types of cells such glioma cells/astrocytes,[41,63,64] skin carcinoma cells/keratinocytes[65] and melanoma cells/melanocytes,[51] has not been mechanistically explained yet, but can be mostly attributed to different cannabinoid receptor-triggered intracellular signaling events in cancer versus non-cancer cells rather than to different expression patterns of cannabinoid receptors between both kinds of cells.[10,19,64]

An additional characteristic of cannabinoids, which may have important clinical implications, is their safety. Cannabinoid-based medicines have been proven very safe in thousands of patients enrolled in multiple clinical trials along the last years and in the cancer patients that use them for the management of pain, nausea and vomiting.[66–68] Moreover, the safety of THC on recurrent glioblastoma multiforme patients was confirmed in a pilot clinical trial.[69] In all these cases, the most reported side effects were mild/moderate dizziness and fatigue.[70]

The most realistic approach to introduce new therapeutic agents in clinical oncology is their combination with standard treatments. There is preclinical evidence showing that the combination of cannabinoids with other established anticancer agents not only does not have negative effects but, instead, induces a synergistic action. For example, temozolomide (the standard therapy for glioma patients) exerted an anti-tumor effect in animal models that was profoundly improved by combination with cannabinoids.[71] Of interest, temozolomide/cannabinoid combinations were very effective in reducing the growth of temozolomide-resistant glioma cell lines.[71] Combination of cannabinoids with other anticancer agents such as gemcitabine, paclitaxel and 5-fluorouracil had synergistic inhibitory effects on the proliferation of cultured pancreatic,[72] gastric[73] and colorectal[74] cancer cells, respectively.

As mentioned above, CBD induces marked anti-tumor actions in models of triple-negative breast cancer and other cancers. The addition of this particular compound to potential cannabinoid-based anticancer therapies would have additional benefits. First, it has been reported that CBD enhances the activity of THC in inhibiting the growth of glioma cells in culture[71,75] and in xenografts,[71] so it is tempting to speculate that this could also be the case for breast cancer. Second, as mentioned above, CBD does not bind with significant affinity to CB_1 receptors and, therefore, it does not exert psychoactivity[25] – moreover, it may attenuate some of the psychoactive effects of THC.[38] Third, CBD exerts by itself a plethora of therapeutic effects [including anxiolytic, antipsychotic, antiepileptic, analgesic, anti-inflamatory, anti-ischemic, neuroprotective and antiemetic effects[38]] in animal models, some of which might be positive for cancer patients.

Conclusions

There is compelling evidence showing that cannabinoids have anti-tumor activity in preclinical models of breast cancer. These data come not only from cell culture systems but also from more complex and clinically relevant animal models. This anti-tumor action is produced by the blockade of several hallmarks of cancer (sustained cancer cell proliferation, metastasis and angiogenesis) rather than by the targeting of a unique process, and the compounds are not only effective but safe. In order to take the next steps towards potential clinical trials our knowledge on several issues should be improved. For example, although the three histopathological subtypes of breast cancer seem to respond to cannabinoids, it would be important to define which the most appropriate patient population for cannabinoid-based therapies is. To date, the most solid preclinical evidence (because of the robustness of the models used and the mechanistic information obtained) points to HER2-positive and triple-negative tumors. Another important question is which cannabinoid/s is/are the best to be tested in patients. In our opinion, the most reasonable candidate would be adding to a standard chemotherapy or immunotherapy a mixture of a cannabinoid targeting CB_1 and/or CB_2 receptors plus CBD. This combination would have the advantage of two cannabinoid compounds acting through different mechanisms of action that would theoretically produce (as it has been demonstrated in mice bearing gliomas) a cooperating anti-tumor effect. In addition, the presence of CBD in the drug cocktail would possibly attenuate non-desired effects of psychoactive cannabinoids and would provide some of the palliative effects that this compound exerts *per se*.

Conflict of interest

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