Minireview

Receptor-dependent and receptor-independent endocannabinoid signaling: A therapeutic target for regulation of cancer growth

Rukiyah Van Dross [a], Eman Soliman [a], Shalini Jha [b], Travious Johnson [b], Somnath Mukhopadhyay [b],*

[a] Department of Pharmacology & Toxicology at Brody School of Medicine, East Carolina University Greenville, NC 27834 USA
[b] Neuroscience & Cancer Research Program, Biomedical Biotechnology Research Institute, North Carolina Central University, Durham, NC 27707 USA

ABSTRACT

The endocannabinoid system comprises the G-protein coupled CB1 cannabinoid receptor (CB1R) and CB2 cannabinoid receptor (CB2R), their endogenous ligands (endocannabinoids), and the enzymes responsible for their synthesis and catabolism. Recent works have revealed several important interactions between the endocannabinoid system and cancer. Moreover, it is now well established that synthetic small molecule cannabinoid receptor agonist acting on either CB1R or CB2R or both exerts anti-cancer effects on a variety of tumor cells. Recent results from many laboratories reported that the expression of CB1R and CB2R in prostate cancer, breast cancer, and many other cancer cells is higher than that in corresponding non-malignant tissues. The mechanisms by which cannabinoids acting on CB1R or CB2R exert their effects on cancer cells are quite diverse and complex. Further, several studies demonstrated that some of the anti-proliferative and apoptotic effects of cannabinoids are mediated by receptor-independent mechanisms. In this minireview we provide an overview of the major findings on the effects of endogenous and/or synthetic cannabinoids on breast and prostate cancers. We also provide insight into receptor independent mechanisms of the anti-cancer effects of cannabinoids under in vitro and in vivo conditions.

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Introduction

Cannabinoids are a class of pharmacologic compounds that offer potential therapeutic applications as antitumor drugs based on their ability to inhibit tumor cell proliferation and survival. Selective agonists and antagonists of CB1R and CB2R, inhibitors of endocannabinoid hydrolysis, and cannabinoid analogs have been utilized to probe the pathways involved in the effects of the endocannabinoid system on cancer cell apoptosis, proliferation, migration, adhesion, and invasion. Emerging evidence suggests that cannabinoid compounds exert their effect on tumor cells in a receptor-dependent and a receptor-independent manner. Here, we briefly review recent works which suggest that (a) CB1/CB2 receptors are possible drug targets for breast and prostate

* Corresponding author at: Neuroscience & Cancer Research Program, Biomedical Biotechnology Research Institute, North Carolina Central University, 700 George Street, Durham, NC 27707 United States. Tel.: +1 919 530 7762; fax: +1 919 530 7760.
E-mail address: smukhopadhyay@nccu.edu (S. Mukhopadhyay).

cancers and (b) cannabinoid receptor independent pathways which compose or interact with the endocannabinoid system may also serve as anti-tumor drug targets.

Cannabinoids and breast cancer

Elevated expression of CB1R and CB2R in different breast cancer tissue/cell lines has been well described in many reports (Caffarel et al., 2010). Immunoreactivity for CB1R and CB2R was found in 28% and 72%, respectively, of human breast tumor tissues (Qamri et al., 2009; Caffarel et al., 2010). In addition, a significant correlation between CB2R and ErbB2 expressions was found as 91% of the ErbB2-positive tumor tissue expressed CB2R. In contrast, no correlation between CB1R and ErbB2 expressions was found and non-tumor breast tissue expressed little CB1R or CB2R (Caffarel et al., 2010).

Under in vitro conditions, anandamide inhibits cell proliferation in a CB1R-dependent manner (Bisogno et al., 1998; De Petrocellis et al., 1998; Melck et al., 1999, 2000). Likewise 2-AG, oleamide and arvanil inhibited cell proliferation in a CB1R (Bisogno et al., 1998; Melck et al., 2000) but not in a CB2R-dependent manner (Melck et al., 2000). In contrast, the mixed CB1/CB2 agonist WIN55,212-2 and the CB2R agonist JWH133 inhibited cell proliferation (McAllister et al., 2007; Qamri et al., 2009) and migration (McAllister et al., 2007; Qamri et al., 2009) in a CB2 receptor-dependent manner. Other mixed CB1/CB2 agonist including CP55 940 (McAllister et al., 2007) and HU-210 (De Petrocellis et al., 1998) and CB1R antagonists SR141716 (Sarnataro et al., 2006) inhibited cell proliferation.

In in vivo studies, Δ^9-THC reduced tumor growth and metastasis along with cell proliferation and angiogenesis in mice injected with various breast cancer cell lines (Caffarel et al., 2010). This inhibition of cell proliferation was mediated by CB2R, but not by CB1R (Caffarel et al., 2010). Further, in mice injected with different breast cancer cell lines CB2R agonist JWH133 reduced tumor size, decreased lung metastases and inhibited cell proliferation and angiogenesis (Qamri et al., 2009; Caffarel et al., 2010). However, in CB-17 immunodeficient mice injected with MDA-MB-231 cells, mixed CB1/CB2 agonist WIN55,212-2 reduced tumor size, and the number and size of lung metastases, and inhibited cell proliferation and angiogenesis in a CB1/CB2 receptor dependent manner (Qamri et al., 2009).

Cannabinoids and prostate cancer

It has recently been shown that the levels of CB1R and CB2R expressions are higher in prostate cancer cells as compared to normal prostate epithelial cells (see review by Guindon and Hohmann, 2011 and Hermanson and Marnett, 2011). Sarfaraz et al. (2005, 2006) showed that WIN-55,212-2 (WIN; CB1/CB2 agonist) treatment of androgen-responsive LNCaP cells resulted in a dose- and time-dependent inhibition of cell proliferation with a concomitant induction of apoptosis. WIN treatment also decreased prostate-specific antigen (PSA), and androgen receptor mRNA and protein expression. WIN-55,212-2-induced cell cycle arrest was associated with a sustained activation of ERK1/2 (Sarfaraz et al., 2006). These responses were blocked by CB1 and CB2 receptor antagonists indicating the involvement of both receptors. In a later study, Olea-Herrero and colleagues showed that methanandamide (MET), as well as the CB2R specific agonist JWH015 significantly inhibited the androgen-insensitive prostate cancer PC-3 cell proliferation in a CB2R antagonist-sensitive manner (Olea-Herrero et al., 2009). CB2R knockdown blocked this response confirming the involvement of CB2R in this anti-proliferative effect. Furthermore, the authors found that JWH015 treatment triggered de novo synthesis of ceramide in PC3 cells, which was implicated in cannabinoid-induced cell death. Similar to these findings earlier studies by Mimeault et al. (2003) also showed that in androgen-sensitive LNCaP and androgen-insensitive PC3 and DU145 cells the endogenous cannabinoid anandamide

produced apoptotic/necrotic responses that were potentiated by the acidic ceramidase inhibitor, N-oleoylethanolamine and inhibited by the specific ceramide synthetase inhibitor, fumonisin B1 indicating the role of cellular ceramide in these cytotoxic responses (Mimeault et al., 2003). Similar to anandamide, 2-arachidonoyl glycerol (2-AG) and its metabolically stable analog noladin ether have also been shown to inhibit invasion of androgen-insensitive prostate cancer cells.

A recent study by Olea-Herrero and colleagues showed that chronic treatment with CB2R agonist JWH015 significantly reduced PC3 tumor growth in a nude mice xenograft model (Olea-Herrero et al., 2009). Collectively the results from these studies suggest that CB1 or CB2 receptor agonist produced a significant decrease in prostate cancer cell proliferation under in vitro and in vivo conditions.

Cannabinoid receptor independent anti-cancer mechanisms

Recently several studies showed that cannabinoid-mediated cytotoxicity can also occur in a receptor-independent manner. In this section, we discuss the involvement of signaling systems implicated in cannabinoid receptor independent cytotoxic effects in tumor tissues and in various cancer cell lines.

Fatty acid amide hydrolase (FAAH) in cancer

FAAH is a serine hydrolase that metabolizes N-acylethanolamines including AEA, OEA and PEA to fatty acids plus ethanolamine (Cravatt et al., 1996, 2001). FAAH inhibitors prevent N-acylethanolamine degradation (Fegley et al., 2005) thereby enhancing their therapeutic effects including the reduction of pain and inflammation (reviewed in Saario and Laitinen, 2007). A recent report showed that FAAH is overexpressed in prostate cancer cells and that the elevated FAAH expression may correlate with poor patient prognosis and outcome (Thors et al., 2010). Another study demonstrated that the selective FAAH inhibitor, URB597, prevented AEA degradation and also enhanced AEA-mediated cytotoxicity in neuroblastoma cells (Hamtiaux et al., 2011). Although CB1R, TRPV1, PPAR-α, PPAR-γ, and GPR55 were expressed in these cells, selective receptor antagonists were unable to block cell death caused by the co-administration of AEA and URB597. However, the cytotoxicity produced by the combined administration of AEA and URB597 could be reversed by disrupting cell membrane-associated lipid rafts.

Monoacylglycerol lipase (MAGL) in cancer

Monoacylglycerols (MAGs) such as 2-AG, are metabolized to free fatty acids (FFAs) and glycerol by MAGL. MAGL and pro-tumorigenic FFAs were found to be elevated and anti-survival MAGs were downregulated in aggressive compared to non-aggressive tumor cell lines (Nomura et al., 2010, 2011). Blockade of MAGL activity with JZL184 or with selective shRNA suppressed FFA production, tumor cell migration, and tumor invasion, and decreased tumor volume. In contrast, overexpression of MAGL in non-aggressive tumor cells caused an increase in FFA synthesis, tumor cell migration, invasion, and tumor volume. These responses were not blocked by CB1R or CB2R antagonist (Nomura et al., 2010). Further, cannabinoid receptors did not regulate the anti-tumor activity observed during MAGL inhibition in ovarian, melanoma, and breast cancer cells (Nomura et al., 2010).

Endocannabinoids and cycloxygenase-2 (COX-2) in cancer

COX-2 is an enzyme that converts arachidonic acid to prostaglandins, prostacyclins and thromboxanes. COX-2 and prostaglandin E are commonly overexpressed in epithelial cancers including those found in the colon, lung, breast, and skin. In addition to metabolizing arachidonic acid, COX-2 catalyzes the conversion of AEA and 2-AG to ethanolamine-conjugated and glycerol-conjugated prostaglandins,

respectively (Yu et al., 1997; Kozak et al., 2002). Because these endocannabinoid-derived prostaglandins do not bind cannabinoid or prostaglandin receptors (Matias et al., 2004) increasing interest has developed in determining if these bioactive lipids mediate the cytotoxic effects of endocannabinoids.

In colorectal carcinoma cells with elevated COX-2 expression, treatment with AEA resulted in increased E-series prostaglandin synthesis and cell death (Patsos et al., 2005, 2010). Selective inhibition or siRNA-mediated downregulation of COX-2 partially reversed AEA-mediated cytotoxicity and this response could not be blocked by CB1 or CB2 receptor antagonist.

Recently we showed that AEA-induced cytotoxicity was mediated by the production of proapoptotic, J-series prostaglandins in tumorigenic keratinocytes that overexpress COX-2 (Van Dross, 2009; Kuc et al., 2012). In addition, resistance to AEA-induced cytotoxicity was observed in non-tumorigenic keratinocytes with low basal COX-2 expression however, these cells underwent cell death when transfected with an expression plasmid containing COX-2. Blockade of AEA degradation by inhibiting FAAH increased J-series prostaglandin synthesis and apoptosis. Also, the cytotoxic effect of AEA was not blocked by CB1R, CB2R or TRPV1 antagonists (Van Dross, 2009). Thus, AEA-induced cell death in tumor cells which overexpress COX-2 appears to be caused by the conversion of AEA to cytotoxic prostaglandins (Van Dross, 2009; Kuc et al., 2012; Patsos et al., 2005, 2010).

It has also been shown that the non-degradable analog of AEA, R(+)-methanandamide [R(+)-MA] induced cellular apoptosis in neuroglioma cells via COX-2 (Ramer et al., 2001). R(+)-MA caused an increase in COX-2 expression and arachidonic acid-derived PGE2 synthesis through upregulation of Erk and p38 kinases. These responses were not reversed in the presence of CB1, CB2, or TRPV1 receptor antagonists.

Endocannabinoids and lipid rafts in cancer

Lipid rafts are dynamic cellular membrane domains enriched in cholesterol and sphingolipids (Jin et al., 2011). These microdomains serve to concentrate and organize signaling proteins which in turn regulate cellular behavior. Several studies indicate that lipid rafts transmit lethal cannabinoid signals in tumor cells (DeMorrow et al., 2007; Sarker and Maruyama, 2003; Scuderi et al., 2011; Bari et al., 2005). AEA increased ceramide production and Fas/FasL localization to lipid rafts leading to cell death in cholangiocarcinoma cells (DeMorrow et al., 2007). In this report, AEA-mediated cell death was not reversed with antagonist of CB1R or CB2R. In a different study, it was demonstrated that AEA-mediated disruption of lipid rafts blocked the induction of oxidative stress and apoptosis in various cell lines (Sarker and Maruyama, 2003). This process did not require CB1R, CB2R, or TRPV1 since AEA-induced cell death was not blocked with selective receptor antagonist or in cells devoid of these receptors. Scuderi and colleagues also showed that WIN55,212-2 caused lipid raft-mediated cell death in cultured melanoma cells (Scuderi et al., 2011) in a CB1R or CB2R independent manner.

Together these findings show that various cannabinoids induce cell death by modulating the composition and integrity of lipid rafts through a process which may occur in the absence or presence of cannabinoid receptor signaling.

Conclusion

The identification of effective treatments to manage and improve cancer therapy is of paramount importance. Selective inhibition of cannabinoid receptors offers potential for the treatment of many cancers including prostate and breast. In addition, several published works show that other components of the endogenous cannabinoid system may serve as drug targets since cannabinoids display anticancer effects independent of cannabinoid receptors.

References

Bari M, Battista N, Fezza F, Finazzi-Agro A, Maccarrone M. Lipid rafts control signaling of type-1 cannabinoid receptors in neuronal cells. Implications for anandamide-induced apoptosis. J Biol Chem 2005;280:12212–20.

Bisogno T, Katayama K, Melck D, Ueda N, De Petrocellis L, Yamamoto S, et al. Biosynthesis and degradation of bioactive fatty acid amides in human breast cancer and rat pheochromocytoma cells — implications for cell proliferation and differentiation. Eur J Biochem 1998;254:634–42.

Caffarel MM, Andradas C, Mira E, Pérez-Gómez E, Cerutti C, Moreno-Bueno G, et al. Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition. Mol Cancer 2010;9:196–206.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384:83–7.

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci U S A 2001;98: 9371–6.

DeMorrow S, Glaser S, Francis H, Venter J, Vaculin B, Vaculin, et al. Opposing actions of endocannabinoids on cholangiocarcinoma growth: recruitment of Fas and Fas ligand to lipid rafts. J Biol Chem 2007;282:13098–113.

De Petrocellis L, Melck D, Palmisano A, Bisogno T, Laezza C, Bifulco M, DiMarzo V. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. Proc Natl Acad Sci U S A 1998;95(837):5-80.

Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G, et al. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. J Pharmacol Exp Ther 2005;313:352–8.

Guindon J, Hohmann AG. The endocannabinoid system and cancer: therapeutic implication. Br J Pharmacol 2011;163:1447–63.

Hamtiaux L, Hansoulle L, Dauguet N, Muccioli GG, Gallez B, Lambert DM. Increasing antiproliferative properties of endocannabinoids in N1E-115 neuroblastoma cells through inhibition of their metabolism. PLoS One 2011;6:e26823.

Hermanson DJ, Marnett LJ. Cannabinoids, endocannabinoids, and cancer. Cancer Metastasis Rev 2011;30:599–612.

Jin S, Zhou F, Katirai F, Li PL. Lipid raft redox signaling: molecular mechanisms in health and disease. Antioxid Redox Signal 2011;15:1043–83.

Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, Weinander R, et al. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem 2002;277:44877–85.

Kuc C, Jenkins A, Van Dross RT. Arachidonoyl ethanolamide (AEA)-induced apoptosis is mediated by J-series prostaglandins and is enhanced by fatty acid amide hydrolase (FAAH) blockade. Mol Carcinog 2012;51:139–49.

Matias I, Chen J, De PL, Bisogno T, Ligresti A, Fezza F, et al. Prostaglandin ethanolamides (prostamides): in vitro pharmacology and metabolism. J Pharmacol Exp Ther 2004;309:745–57.

McAllister SD, Christian RT, Horowitz MP, Garcia A, Desprez PY. Cannabidiol as a novel inhibitor of Id-1 gene expression in aggressive breast cancer cells. Mol Cancer Ther 2007;6:2921–7.

Melck D, Rueda D, Galve-Roperh I, De Petrocellis L, Guzmán M, Di Marzo V. Involvement of the cAMP/protein kinase A pathway and of mitogen-activated proteinkinase in the anti-proliferative effects of anandamide in human breast cancer cells. FEBS Lett. 1999;463:235–40.

Melck D, De Petrocellis L, Orlando P, Bisogno T, Laezza C, Bifulco M, et al. Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. Endocrinology 2000;2000(141):118–26.

Mimeault M, Pommery N, Wattez N, Bailly C, Hénichart JP. Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. Prostate 2003;56:1-12.

Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 2010;140: 49–61.

Nomura DK, Lombardi DP, Chang JW, Niessen S, Ward AM, Long JZ, et al. Monoacylglycerol lipase exerts dual control over endocannabinoid and fatty acid pathways to support prostate cancer. Chem Biol 2011;18:846–56.

Olea-Herrero N, Vara D, Malagarie-Cazenave S, Diaz-Laviada I. Inhibition of human tumour prostate PC-3 cell growth by cannabinoids R(+)-methanandamide and JWH-015: involvement of CB2. Br J Cancer 2009;101:940–50.

Patsos HA, Hicks DJ, Dobson RR, Greenhough A, Woodman N, Lane JD, et al. The endogenous cannabinoid, anandamide, induces cell death in colorectal carcinoma cells: a possible role for cyclooxygenase 2. Gut 2005;54:1741–50.

Patsos HA, Greenhough A, Hicks DJ, Al KM, Collard TJ, Lane JD, et al. The endogenous cannabinoid, anandamide, induces COX-2-dependent cell death in apoptosis-resistant colon cancer cells. Int J Oncol 2010;37:187–93.

Qamri Z, Preet A, Nasser MW, Bass CE, Leone G, Barsky SH, et al. Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. Mol Cancer Ther 2009;8:3117–29.

Ramer R, Brune K, Pahl A, Hinz B. R(+)-methanandamide induces cyclooxygenase-2 expression in human neuroglioma cells via a non-cannabinoid receptor-mediated mechanism. Biochem Biophys Res Commun 2001;286:1144–52.

Saario SM, Laitinen JT. Therapeutic potential of endocannabinoid-hydrolysing enzyme inhibitors. Basic Clin Pharmacol Toxicol 2007;101:287–93.

Sarfaraz S, Afaq F, Adhami VM, Mukhtar H. Cannabinoid receptor as a novel target for the treatment of prostate cancer. Cancer Res 2005;65:1635–41.

Sarfaraz S, Afaq F, Adhami VM, Malik A, Mukhtar H. Cannabinoid receptor agonist-induced apoptosis of human prostate cancer cells LNCaP proceeds through sustained activation of ERK1/2 leading to G1 cell cycle arrest. J Biol Chem 2006;281:39480–91.

Sarker KP, Maruyama I. Anandamide induces cell death independently of cannabinoid receptors or vanilloid receptor 1: possible involvement of lipid rafts. Cell Mol Life Sci 2003;60:1200–8.

Sarnataro D, Pisanti S, Santoro A, Gazzerro P, Malfitano AM, Laezza C, et al. The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits human breast cancer cell proliferation through a lipid raft-mediated mechanism. Mol Pharmacol 2006;70:1298–306.

Scuderi MR, Cantarella G, Scollo M, Lempereur L, Palumbo M, Saccani-Jotti G, et al. The antimitogenic effect of the cannabinoid receptor agonist WIN55212-2 on human melanoma cells is mediated by the membrane lipid raft. Cancer Lett 2011;310:240–9.

Thors L, Bergh A, Persson E, Hammarsten P, Stattin P, Egevad L, et al. Fatty acid amide hydrolase in prostate cancer: association with disease severity and outcome, CB1 receptor expression and regulation by IL-4. PLoS One 2010;5:e12275.

Van Dross RT. Metabolism of anandamide by COX-2 is necessary for endocannabinoid-induced cell death in tumorigenic keratinocytes. Mol Carcinog 2009;48:724–32.

Yu M, Ives D, Ramesha CS. Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. J Biol Chem 1997;272:21181–6.