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Receptor-dependent and Receptor-independent Endocannabinoid Signaling: A Therapeutic Target for Regulation of Cancer Growth

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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 exert 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 are higher than 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 minreview we provide an overview of the major findings on the effects of endogenous and/or synthetic cannabinoids on breast and prostate cancer. We also provide insight into receptor independent mechanisms of the anti-cancer effects of cannabinoids under in vitro and in vivo conditions.

Keywords

Cannabinoids; Endocannabinoids; Cancer; Tumor cells; Receptor-dependent; Receptor-independent

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Introduction

Cannabinoids are a class of pharmacologic compounds that offer potential therapeutic applications as antitumor drugs based on their ability 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 receptor-independent manner. Here, we briefly review recent works which suggest that (a) CB1/CB2 receptors are possible drug targets for breast and prostate cancer 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 expression was found as 91% of the ErbB2-positive tumor tissue expressed CB2R. In contrast, no correlation between CB1R and ErbB2 expression 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-depended 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 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 migraton (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 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, 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 expression are higher in prostate cancer cells as compared to normal prostate epithelial cells (see review Guindon and Hohmann, 2011; Hermanson and Marnett, 2011). Sarafraz and colleagues (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-Heraro and colleagues showed that methanandamide (MET), as well as the CB2R specific agonist JWH015 significantly inhibited 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-prolifeartive 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 Mimeallt and colleagues (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 has also been shown to inhibit invasion of androgen-insensitive prostate cancer cells.

A recent study by Olea-Heraro 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 results from these studies suggest that CB1 or CB2 receptor agonists 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 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, 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 antagonists (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).

Endocannbinoids 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, Jenkins, and Van Dross, 2012;Patsos et al., 2010; Pastos et al., 2005).

It has also been shown that the non-degradable analogue 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 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.

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