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REVIEW

The endocannabinoid system and cancer: therapeutic implication

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The endocannabinoid system is implicated in a variety of physiological and pathological conditions (inflammation, immunomodulation, analgesia, cancer and others). The main active ingredient of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), produces its effects through activation of CB_1 and CB_2 receptors. CB_1 receptors are expressed at high levels in the central nervous system (CNS), whereas CB_2 receptors are concentrated predominantly, although not exclusively, in cells of the immune system. Endocannabinoids are endogenous lipid-signalling molecules that are generated in the cell membrane from phospholipid precursors. The two best characterized endocannabinoids identified to date are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Here we review the relationship between the endocannabinoid system and anti-tumour actions (inhibition of cell proliferation and migration, induction of apoptosis, reduction of tumour growth) of the cannabinoids in different types of cancer. This review will focus on examining how activation of the endocannabinoid system impacts breast, prostate and bone cancers in both *in vitro* and *in vivo* systems. The therapeutic potential of cannabinoids for cancer, as identified in clinical trials, is also discussed. Identification of safe and effective treatments to manage and improve cancer therapy is critical to improve quality of life and reduce unnecessary suffering in cancer patients. In this regard, cannabis-like compounds offer therapeutic potential for the treatment of breast, prostate and bone cancer in patients. Further basic research on anti-cancer properties of cannabinoids as well as clinical trials of cannabinoid therapeutic efficacy in breast, prostate and bone cancer is therefore warranted.

LINKED ARTICLES

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Abbreviations

2-AG, 2-arachidonoylglycerol; AC, adenylyl cyclase; ACEA, arachidonyl-2′-chloroethylamide; AEA, anandamide; AKT, protein kinase B; AR, androgen receptor; ATP, adenosine triphosphate; Bax, pro-apoptotic protein; Bcl2, anti-apoptotic protein; brca1, breast cancer susceptibility gene product; cAMP, cyclic adenosine monophosphate; CBD, cannabidiol; CB, cannabinoid; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CBN, cannabinol; Cdc2, p34 cyclin-dependent kinase 1; CDK, cyclin-dependent kinases; CNS, central nervous system; DALN, desacetyllevonantradol; Δ^9-THC, delta 9-tetrahydrocannabinol; DRG, dorsal root ganglion; EGF, epidermal growth factor receptor; EPEA, eicosapentaenoyl ethanolamide; ERK, extracellular regulated kinase; FAAH, fatty-acid amide hydrolase; GPR55, G-protein-coupled receptor 55; H_2O_2, hydrogen peroxide; p27/KIP1, cyclin kinase inhibitor; p38MAPK, p38 mitogen-activated protein kinase; PRL, prolactin receptor; MET, methanandamide; MGL or MAGL, monoacylglycerol lipase; OTFP, 3-octylthio-1,1,1-trifluoropropan-2-one; p53, p53 protein; p21ras, p21 ras protein; PEA, palmitoylethanolamide; PI3K, phosphatidyl inositol 3 kinase; PKA, protein kinase A; PKB, protein kinase B; PRL, prolactin receptor; PSA, prostate-specific antigen; Raf-1, protein Raf-1; RT-PCR, reverse transcriptase polymerase chain reaction; Trk, high-affinity nerve growth factor receptor; TRPV1, transient receptor potential cation channel V1

Introduction

Cancer resulted in approximately 7.6 million deaths worldwide in 2008 (Ferlay *et al.*, 2010). An estimated 12.7 million new cancer cases were diagnosed in 2008 alone (Ferlay *et al.*, 2010). Worldwide, an estimated 1.38 million women and 914 000 men were diagnosed with breast and prostate cancer, respectively, in 2008 (Ferlay *et al.*, 2010). If this trend continues, cancer will overtake heart disease and become the predominant cause of death (Heron *et al.*, 2009). Effective treatment and management of cancer is critical for cancer patients but the development of safe and effective treatments that improve cancer therapy remains an unmet need. Cannabinoids and modulators of the endocannabinoid system have recently been shown to produce anti-tumour actions (reduction of inflammation, cell proliferation and cell survival properties) in different models of cancer. The present review focuses on breast, prostate and bone cancer in which links to the endocannabinoid system have been studied. More work is necessary to determine whether pharmacotherapies targeting the endocannabinoid system improve the treatment of cancer in patients.

Cannabinoids are implicated in a variety of physiological and pathological conditions including inflammation, immunomodulation, analgesia, cancer and others (for reviews Pacher *et al.*, 2006; Di Marzo, 2008). The main active ingredient of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), produces its effects through activation of G-protein-coupled CB_1 (Matsuda *et al.*, 1990; Ledent *et al.*, 1999; Zimmer *et al.*, 1999) and CB_2 (Munro *et al.*, 1993; Buckley *et al.*, 2000) receptors. Endocannabinoids are endogenous lipid-signalling molecules that are generated in the cell membrane from phospholipid precursors. They bind and activate one or more cannabinoid receptor subtypes, thus producing cannabimimetic properties (for reviews Piomelli, 2005; Di Marzo, 2006). The two best studied endocannabinoids isolated to date are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Anandamide is hydrolysed by the enzyme fatty-acid amide hydrolase (FAAH) whereas 2-AG is degraded predominantly, although not exclusively, by monoacylglycerol lipase (MGL) (for reviews Pacher *et al.*, 2006; Jhaveri *et al.*, 2007; Guindon and Hohmann, 2009; Wang and Ueda, 2009). Thus, enzymes catalysing endocannabinoid hydrolysis represent potential new targets for cancer pharmacotherapies. In this review, drug and molecular target nomenclature conforming to *British Journal of Pharmacology* Guide to Receptors and Channels (Alexander *et al.*, 2009) has been employed. Here, we will refer to effects of cannabinoids blocked by CB_1 antagonists (SR141716A, AM251) as being CB_1-mediated and effects blocked by CB_2 antagonists (SR144528, AM630) as CB_2-mediated.

Cancer is marked by uncontrolled cell division and cell death emerging from cumulative damage of important regulatory genes. Multiple genes likely need to be damaged in order for a cancer to grow and develop the ability to spread (i.e. metastasize). Cancers may be hereditary and/or caused by external (tobacco, chemicals, radiation, infectious organisms and others) and/or internal (inherited mutations, hormones, mutations from metabolism and others) factors. This review will focus on uncovering connections between the endocannabinoid system and breast, prostate and bone cancers with an emphasis on understanding how these connections could be exploited for their therapeutic anti-cancer potential.

Animal models of cancer have been developed to experimentally assess pathophysiological mechanisms implicated in the analogous clinical syndrome. These models provide insight into pathophysiological processes of the disease state and elucidate mechanisms of action that may be targeted by drug discovery efforts aimed at identifying novel therapeutics. These models thus permit preclinical evaluation and validation of therapeutic efficacy of new pharmacotherapies.

Anti-proliferative properties of cannabis compounds were first identified 35 years ago. Here, it was first shown that administration of Δ^9-THC inhibits lung adenocarcinoma cell growth *in vivo* (i.e. after oral administration in mice) and *in vitro* (Munson *et al.*, 1975; Carchman *et al.*, 1976). It took more that two decades before the potential anti-tumour properties associated with a cannabinoid were investigated further. In the past 14 years, an emerging body of research, primarily employing *in vitro* models of different cancers, has helped elucidate the mechanisms through which cannabinoids and the endocannabinoid tumour system influences cancer cell proliferation, migration and apoptosis (i.e. programmed cell death).

The mechanisms through which cannabinoids/cannabinoids receptors impact proliferation, migration and apoptosis of cancer cells are quite complex and our understanding of these processes remain incomplete. Moreover, these mechanisms differ in different types of cancer, and both pro- and anti-apoptotic effects of cannabinoids have been reported. A schematic representation of the major signalling pathways that are implicated in the activation of different cannabinoid receptor subtypes through their agonists and their involvement in these processes is summarized in Figure 1.

Several mechanisms are likely to underline the pro-apoptotic effects of cannabinoids and explain their anti-cancer effects. Cannabinoids induce de novo synthesis of ceramides, a family of lipid molecules composed of sphingosine and a fatty acid, found in the cell membrane. Synthesis of ceramide occurs via activation of the enzyme ceramide synthase and leads to downstream activation of an extracellular regulated kinase (ERK) signalling cascade. This process results in cell cycle arrest and apoptosis. Activation of either CB_1 or CB_2 receptors triggers the ceramide-ERK signalling pathway to promote apoptosis (Kogan, 2005; Sarfaraz *et al.*, 2006, 2008) (Figure 1). The increase in ceramide can also activate the p38 mitogen-activated protein kinase (p38MAPK) pathway which can lead to apoptosis through multiple mechanisms (i.e. through activation of cysteine proteases (i.e. caspases) or through cytochrome C release from mitochondria). The sustained activation of ERK also promotes the induction of cyclin kinase inhibitor (p27/KIP1) which modulates regulatory molecules of the cell cycle (cyclins, cdks) resulting in cell cycle arrest and apoptosis (Kogan, 2005; Sarfaraz *et al.*, 2006, 2008) (Figure 1). Cell cycle arrest involves the up-regulation of the p53 protein which will differentially alter levels of pro- and anti-apoptotic proteins (i.e. increase the levels of the pro-apoptotic protein Bax and lower the levels of the anti-apoptotic protein Bcl2, respectively, thereby shifting the ratio towards Bax) which ulti-

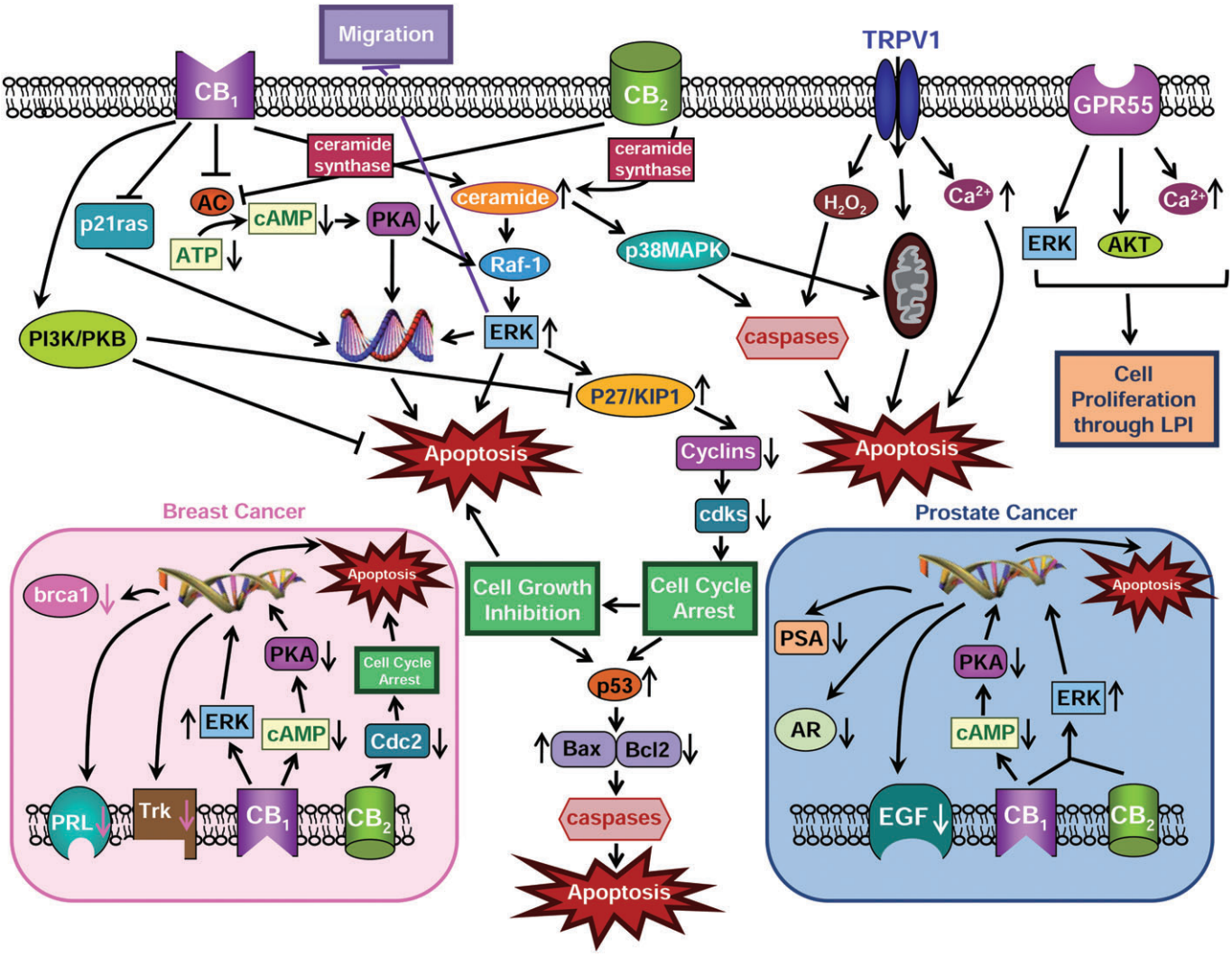

Figure 1

Schematic representation of different mechanisms/signalling pathways through which cannabinoids impact apoptosis, proliferation and migration. AC, adenylyl cyclase; AKT, protein kinase B; AR, androgen receptor; ATP, adenosine triphosphate; Bax, pro-apoptotic protein; Bcl2, antiapoptotic protein; brca1, breast cancer susceptibility gene product; cAMP, cyclic adenosine monophosphate; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; Cdc2, p34 cyclin-dependent kinase 1; CDK, cyclin-dependent kinases; EGF, epidermal growth factor; ERK, extracellular regulated kinase; GPR55, G protein-coupled receptor 55; H_2O_2, hydrogen peroxide; p27/KIP1, cyclin kinase inhibitor; PRL, prolactin receptor; p53, p53 protein; p21ras, p21 ras protein; PI3K, phosphatidyl inositol 3 kinase; PKA, protein kinase A; PKB, protein kinase B; PSA, prostate-specific antigen; Raf-1, protein Raf-1; Trk, high-affinity nerve growth factor receptor; TRPV1, transient receptor potential cation channel V1.

mately leads to activation of caspases that play an essential role in triggering apoptosis (Sarfaraz *et al.*, 2006). Activation of either CB_1 or CB_2 receptors also inhibits adenylyl cyclase (AC) activity and lowers both cyclic adenosine monophosphate (cAMP) levels and protein kinase A (PKA) activity, thereby causing down-regulation of gene transcription, leading to apoptosis (Guzmán, 2003; Kogan, 2005; Bifulco *et al.*, 2008; Sarfaraz *et al.*, 2008). Activation of transient receptor potential cation channel V1 (TRPV1) receptors also leads to increases in intracellular levels of both hydrogen peroxide (H_2O_2) and/or calcium or the release of cytochrome C from mitochondria, causing apoptosis through both distinct and overlapping mechanisms (Figure 1) (Maccarrone *et al.*, 2000).

Cannabinoid receptor 1 ligands have pro-apoptotic properties, in part, through inhibition of the Ras protein (p21ras) which is involved in inducing DNA synthesis (Bifulco *et al.*, 2001). However, CB_1 receptor activation can also trigger activation of different tumour cascades that are linked to promotion of cancer cell survival and inhibition of apoptosis. Indeed, CB_1 ligands also stimulate the PI3K/PKB tumour pathway, an essential tumour pathway implicated in cell survival. Activation of PI3K/PKB downstream of CB_1 activation may promote apoptosis either directly or through inhibition of p27/KIP1 (Gómez del Pulgar *et al.*, 2000; Sarfaraz *et al.*, 2008). Thus, both pro- and anti-apoptotic effects of cannabinoids have been reported, raising the possibility that a dysregulation of the endocannabinoid system may also

contribute to cancer/tumour development. However, activation of ERK downstream of cannabinoid receptor activation also promotes anti-cancer effects through inhibition of cancer cell migration (Blazquez *et al.*, 2003; Kogan, 2005). More work is necessary to determine the balance between these various mechanisms, and how they modulate cancer *in vivo*.

Phytocannabinoids may also interfere with the ability of lysophosphatidylinositol (LPI), a putative endogenous ligand for G-protein-coupled receptor 55 (GPR55), to promote cancer cell proliferation through activation of the putative novel cannabinoid receptor subtype (Figure 1). Pretreatment of breast and prostate cancer cells with cannabidiol, a major component of marijuana, or with SR141716A (a CB_1 antagonist that also possesses agonist/antagonist properties at GPR55), blocks the ability of LPI to induce cell proliferation through GPR55, thereby producing anti-cancer effects (Piñeiro *et al.*, 2011). Activation of GPR55 by LPI promotes cancer cell proliferation following activation of distinct intracellular tumour cascades [i.e. ERK, AKT and calcium mobilization (Piñeiro *et al.*, 2011)]; these anti-proliferative effects were also blocked using siRNA for GPR55.

Breast cancer

Breast cancer is the most common cancer among women (Ferlay et al., 2010). Genetics, lack of child bearing/breastfeeding, higher hormone levels and iodine deficiency have all been identified as risk factors for developing breast cancer (Madigan *et al.*, 1995). Breast cancer, also known as malignant breast neoplasm, originates from breast tissue, usually from the milk ducts (ductal carcinomas) or the lobules (lobular carcinomas) that supply the ducts with milk (Glass *et al.*, 2007). Breast cancer cells may spread to other organs such as the bones, lungs and lymph nodes (Guise *et al.*, 2010; Pantel and Alix-Panabières, 2010). Recent research identifies a role for the endocannabinoid system in the regulation of tumour growth, induction of apoptosis (programmed cell death) and control of tumour vascularization (angiogenesis) in breast cancer (Grimaldi *et al.*, 2006; Ligresti *et al.*, 2006; Qamri *et al.*, 2009; Caffarel *et al.*, 2010). The phenomenon of angiogenesis is required for tumours to transition from a dormant to malignant state (Fontanini, 2000; Ribatti and Crivellato, 2010).

Expression of cannabinoid receptors in different breast cancer tissue/cell lines has been described. CB_1 expression by immunohistochemistry was detected in 14% of human breast cancer tumour tissue expressing a member of the epidermal growth factor (EGF) family referred to as the ErbB2 tyrosine kinase receptor. No correlation between CB_1 expression and ErbB2 expression was found (Caffarel *et al.*, 2010). CB_1 immunoreactivity was also expressed in 28% of human breast carcinoma (Qamri *et al.*, 2009). CB_1 receptors are also present in different breast cancer cell lines (MCF-7, T-47D, MDA-MB-231, TSA-E1, MDA-MB-468) and in human breast tissues using RT-PCR, immunofluorescence and/or Western blot (Melck *et al.*, 2000; Di Marzo *et al.*, 2001; McKallip *et al.*, 2005; Sarnataro *et al.*, 2005, 2006; Caffarel *et al.*, 2006; Grimaldi *et al.*, 2006; Ligresti *et al.*, 2006; Qamri *et al.*, 2009). By contrast, CB_2 immunoreactivity was detected in 72% of human breast tumour tissue (Caffarel *et al.*, 2010). Interestingly, CB_2 receptors were found in 91% of ErbB2-positive tumour tissue, suggesting a link between CB_2 and ErbB2-expression, but not between CB_1 and ErbB2-expression (Caffarel *et al.*, 2010). In another study, CB_2 receptors immunoreactivity was observed in 35% of human breast carcinoma (Qamri *et al.*, 2009). However, CB_2 receptors are also expressed in different breast carcinoma cell lines (MCF-7, T-47D, MDA-MB-231, MDA-MB-468, EVSA-T, SkBr3) and human breast tissues using RT-PCR, immunofluorescence and/or Western blot (Melck *et al.*, 2000; Di Marzo *et al.*, 2001; McKallip *et al.*, 2005; Ligresti *et al.*, 2006; Qamri *et al.*, 2009; Caffarel *et al.*, 2006, 2010). The putative novel cannabinoid receptor subtype GPR55 was highly expressed in a MDA-MB-231 cell line using RT-PCR, but it is expressed at lower (30-fold) levels in MCF-7 breast carcinoma cell lines using RT-PCR (Ford *et al.*, 2010). Lysophosphatidylinositol (LPI), the putative endogenous ligand for GPR55, also stimulates cell migration and invasion in a MDA-MB-231 cell line and this LPI effect on migration is blocked by pretreatment with cannabidiol (CBD) (Ford *et al.*, 2010). Furthermore, it was also demonstrated that LPI stimulate proliferation and this effect was blocked by CBD (Piñeiro *et al.*, 2011). Moreover, the anandamide hydrolysing enzyme (FAAH) is found in EFM-19 and MCF-7 cancer cell lines using Northern blot analyses (Bisogno *et al.*, 1998) or RT-PCR (Takeda *et al.*, 2008). No study has evaluated whether the 2-AG hydrolysing enzyme MGL is similarly present, or whether changes in enzymes catalysing endocannabinoid synthesis or degradation accompany anti-cancer effects. Nonetheless, the literature suggests that multiple cannabinoid receptor subtypes as well as enzymes catalysing endocannabinoid hydrolysis (i.e. FAAH) show an anatomical distribution appropriate to regulate breast cancer cell proliferation, migration and/or apoptosis.

In vitro studies show that endocannabinoids and cannabinoid-like compounds inhibit proliferation and/or migration and/or induce apoptosis in different breast carcinoma (MCF-7, EFM-19, T-47D, MDA-MB-231, MDA-MB-468, MDA-MB-436, 4T1, TSA-E1, EVSA-T, SkBr3, HTB-126) cell lines. It is important to note that the MDA-MB-231 cell line represents a small proportion of all types of breast cancer; it is highly metastatic and lacks expression of other receptors (oestrogen, progesterone). The phytocannabinoid CBD inhibits cell proliferation (Ruh *et al.*, 1997; Ligresti *et al.*, 2006; McAllister *et al.*, 2007, 2010), increases apoptosis (Ligresti *et al.*, 2006) and reduces migration (McAllister *et al.*, 2007, 2010; Ford *et al.*, 2010) in different cancer cell lines (Table 1). Anti-proliferative effects of CBD are partially reversed by SR144528 (Ligresti *et al.*, 2006), although a possible role for CB_1 cannot be ruled out because its possible contribution was not evaluated. The mechanism of action of CBD in producing these effects is not fully understood and needs to be investigated further. Δ^9-THC also possesses anti-proliferative properties (McAllister *et al.*, 2007; Caffarel *et al.*, 2006, 2008; von Bueren *et al.*, 2008), increases apoptosis (Caffarel *et al.*, 2006) and decreases cancer cell migration (McAllister *et al.*, 2007). Δ^9-THC-induced anti-proliferative and pro-apoptotic properties are mediated by CB_2, but not CB_1 receptors (Caffarel *et al.*, 2006). The lack of effect of Δ^9-THC on CB_1 receptors in EVSA-T cells could be explained by the lack of CB_1 receptors in this cell line. By contrast, some studies have shown that

Table 1

Breast cancer *in vitro* and *ex vivo* studies

Cell line/tissue	Expressing	Apoptosis	Proliferation	Migration	Apoptosis/proliferation/migration mediated by CB_1	Apoptosis/proliferation/migration mediated by CB_2	Reference
MCF-7	–	–	↓ by DALN, CBD	–	–	–	Ruh *et al.*, 1997
EFM-19	FAAH	–	↓ by AEA	–	SR1	–	Bisogno *et al.*, 1998
EFM-19	FAAH	–	↓ by Oleamide	–	SR1	–	Bisogno *et al.*, 1998
MCF-7 EFM-19	–	–	↓ by AEA	–	SR1	–	De Petrocellis *et al.*, 1998
EFM-19	–	–	↓ by 2-AG, MET, HU-210	–	–	–	De Petrocellis *et al.*, 1998
MCF-7 EFM-19	–	–	↓ by AEA	–	–	–	Melck *et al.*, 1999
MCF-7 T-47D	CB_1, CB_2	–	↓ by AEA, arvanil	–	SR1	Not SR2	Melck *et al.*, 2000
MCF-7 T-47D	CB_1, CB_2	–	↓ by 2-AG	–	–	–	Melck *et al.*, 2000
MCF-7	CB_1, CB_2	–	PEA enhance AEA ↓	–	–	Not SR2	Di Marzo *et al.*, 2001
MCF-7	CB_1, CB_2	–	PEA enhance arvanil ↓ PEA enhance HU-210 ↓	–	–	–	Di Marzo *et al.*, 2001
EFM-19	–	–	PEA enhance olvanil ↓	–	–	Not SR2	De Petrocellis *et al.*, 2002
MCF-7	CB_1 low, not CB_2	Δ^9-THC no Δ	–	–	–	–	McKallip *et al.*, 2005
4T1*	Not CB_1 nor CB_2	Δ^9-THC no Δ	–	–	–	–	McKallip *et al.*, 2005
MDA-MB-231	CB_1	–	–	–	–	–	Sarnataro *et al.*, 2005
MCF-7	–	–	↑ by Δ^9-THC, CBD, CBN	–	–	–	Watanabe *et al.*, 2005
EVSA-T	CB_2	↑ by Δ^9-THC	↓ by Δ^9-THC	–	Not SR1 pro	SR2 pro, apo	Caffarel *et al.*, 2006
MCF-7 T-47D MDA-MB-231 MDA-MB-468 SkBr3	–	–	↓ by Δ^9-THC	–	–	–	Caffarel *et al.*, 2006
Breast tumour	CB_1, CB_2	–	–	–	–	–	Caffarel *et al.*, 2006
MDA-MB-231	–	MET no Δ	↓ by MET	↓ by MET	SR1 mig	–	Grimaldi *et al.*, 2006
T-47D	CB_1	–	MET no Δ	–	–	–	Grimaldi *et al.*, 2006
TSA-E1*	CB_1	–	↓ by MET	↓ by MET	SR1 mig	–	Grimaldi *et al.*, 2006
MCF-7	CB_1 weak, CB_2 weak	–	↓ by CBD, CBG, CBC, CBD acid, THC acid	–	–	–	Ligresti *et al.*, 2006
MDA-MB-231	CB_1 weak, CB_2 medium	↑ by CBD	↓ by CBD, CBG, CBC, CBD acid, THC acid	–	–	Partially by SR2 with CBD for pro	Ligresti *et al.*, 2006

Table 1

Continued.

Cell line/tissue	Expressing	Apoptosis	Proliferation	Migration	Apoptosis/proliferation/migration mediated by CB_1	Apoptosis/proliferation/migration mediated by CB_2	Reference
MCF-7 T-47D	CB_1	–	↓ by SR1	–	–	–	Sarnataro *et al.*, 2006
MDA-MB-231	CB_1	SR1 no Δ	↓ by SR1	–	–	–	Sarnataro *et al.*, 2006
MDA-MB-231	–	–	↓ by Δ^9-THC, CBN, WIN-2, CP55,940, CBD	↓ by Δ^9-THC, CBD, WIN-2	–	–	McAllister *et al.*, 2007
MDA-MB-436	–	–	↓ by Δ^9-THC, CBN, WIN-2, CP55,940, CBD	↓ by CBD	–	–	McAllister *et al.*, 2007
HTB-126	–	–	↓ by NPT, NPD	–	SR1 with NPT	–	Burstein and Salmonsen, 2008
EVSA-T	–	–	↓ by Δ^9-THC	–	–	–	Caffarel *et al.*, 2008
MDA-MB-231	–	–	–	↓ by MET	SR1	–	Laezza *et al.*, 2008
MCF-7	FAAH Not CB_1 nor CB_2	–	↑ by Δ^9-THC, CBD, CBN	–	Not SR1 nor AM251 with Δ^9-THC	–	Takeda *et al.*, 2008
MCF7 MCF7-AR1	–	–	↓ by Δ^9-THC	–	–	–	von Bueren *et al.*, 2008
MDA-MB-231	CB_1, CB_2	↑ by JWH-133, WIN-2	↓ by JWH-133, WIN-2	↓ by JWH-133, WIN-2	–	siRNA and WB	Qamri *et al.*, 2009
MDA-MB-468	CB_1, CB_2	–	↓ by JWH-133, WIN-2	↓ by JWH-133, WIN-2	–	–	Qamri *et al.*, 2009
MCF-7	–	–	↑ by Δ^9-THC	–	–	–	Takeda *et al.*, 2009
Breast tumour	CB_1, CB_2	–	–	–	–	–	Caffarel *et al.*, 2010
MDA-MB-231	GPR55	–	–	↓ by CBD	–	–	Ford *et al.*, 2010
MCF-7 MDA-MB-231	–	–	↓ by MET	–	–	–	Laezza *et al.*, 2010
MCF-7 MDA-MB-231	–	–	↓ by CBD	↓ by CBD	–	–	McAllister *et al.*, 2010

Human and murine* cell line/tissue.

↑, increase; ↓, decrease; –, not tested; 2-AG, 2-arachidonoyl glycerol; apo, apoptosis; AEA, anandamide; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CBC, cannabichromene; CBD, cannabidiol; CBD acid; cannabidiol acid; CBG, cannabigerol; CBN, cannabinol; Δ^9-THC, delta 9-tetrahydrocannabinol; DALN, desacetyllevonantradol; FAAH, fatty-acid amide hydrolase; MET, methanandamide; mig, migration; no Δ, no change; NPD, N-palmitoyl dopamine; NPT, N-palmitoyl tyrosine; PEA, palmitoylethanolamide; pro, proliferation; siRNA, silencing RNA; SR1, SR141716A; SR2, SR144528; THC acid, tetrahydrocannabinol acid; WB, Western blot; WIN-2, WIN55,212-2.

Δ^9-THC failed to inhibit cell proliferation (Ruh *et al.*, 1997) or induce apoptosis (McKallip *et al.*, 2005). Cannabinol (CBN), a phytocannabinoid that is both a metabolite of Δ^9-THC and a weak agonist at CB_1 and CB_2 receptors, also inhibits cell proliferation (McAllister *et al.*, 2007). Another study provided findings contradictory to these observations; CBD, Δ^9-THC and CBN all stimulated proliferation at low (1 μM) or high (up to 20 μM) concentrations in MCF-7 cells, but there were limitations in the studies which affect data interpretation. For example, these groups failed to demonstrate the expression of CB_1 or CB_2 receptors (Watanabe *et al.*, 2005; Takeda *et al.*, 2008), a finding which conflicts with other studies that show CB_1 and CB_2 receptor expression in the same cell line (Movsesyan *et al.*, 2004; McKallip *et al.*, 2005). Nonetheless, the possibility remains that the combination of phytocannabinoids in cannabis may offer greater therapeutic potential compared with Δ^9-THC or CBD alone.

Endocannabinoids exhibit anti-proliferative properties *in vitro*. Indeed, AEA inhibits cell proliferation (Bisogno *et al.*, 1998; De Petrocellis *et al.*, 1998; Melck *et al.*, 1999, 2000) which is mediated by CB_1 (Bisogno *et al.*, 1998; De Petrocellis *et al.*, 1998; Melck *et al.*, 2000), but not by CB_2 (Melck *et al.*, 2000) receptors (Table 1). However, CB_2-mediated effects of AEA cannot be discounted because only one study evaluated their possible contribution (Melck *et al.*, 2000). Moreover, 2-AG, oleamide and arvanil all inhibit cell proliferation. The anti-proliferative effects of oleamide and arvanil are inhibited by CB_1 (Bisogno *et al.*, 1998; Melck *et al.*, 2000), but not CB_2 (Melck *et al.*, 2000) receptor antagonists. Interestingly, palmitoylethanolamide (PEA), a fatty-acid amide that does not bind to cannabinoid receptors, enhances the anti-proliferative effects of AEA, arvanil, olvanil and HU-210 (Di Marzo *et al.*, 2001; De Petrocellis *et al.*, 2002), which is suggestive of synergism or an entourage effect (Ben-Shabat *et al.*, 1998; De Petrocellis *et al.*, 2002). Moreover, the enhancement of AEA and olvanil anti-proliferative effects does not involve CB_2 receptors (Di Marzo *et al.*, 2001; De Petrocellis *et al.*, 2002). Methanandamide inhibits both cell proliferation (De Petrocellis *et al.*, 1998; Grimaldi *et al.*, 2006; Laezza *et al.*, 2010) and cell migration (Grimaldi *et al.*, 2006; Laezza *et al.*, 2008). The methanandamide-induced inhibition of cell migration involves CB_1 receptors (Grimaldi *et al.*, 2006). By contrast, the mixed CB_1/CB_2 agonist WIN55,212-2 and CB_2 agonist JWH-133 inhibits both cell proliferation (McAllister *et al.*, 2007; Qamri *et al.*, 2009) and migration (McAllister *et al.*, 2007; Qamri *et al.*, 2009) through a mechanism that requires CB_2 receptor activation, although a possible role for CB_1 was not assessed (Qamri *et al.*, 2009) (Table 1). Surprisingly, both cannabinoid agonists such as CP55 940 (McAllister *et al.*, 2007) and HU-210 (De Petrocellis *et al.*, 1998) and cannabinoid CB_1 antagonists such as SR141716A (Sarnataro *et al.*, 2006) have all been shown to inhibit cell proliferation. Amide derivatives such as N-palmitoyl tyrosine and N-palmitoyl dopamine also possess anti-proliferative properties; in the case of N-palmitoyl tyrosine, effects are mediated by CB_1 receptors (Burstein and Salmonsen, 2008). Cannabigerol, cannabichromene, cannabidiol acid and THC acid (Ligresti *et al.*, 2006) as well as desacetyllevonantradol (Ruh *et al.*, 1997) also inhibit cell proliferation in different breast cancer cell lines (Table 1).

In breast cancer cells, cannabinoid receptor agonists inhibit breast cancer cell proliferation by a down-regulation of high-affinity nerve growth factor (Trk) and prolactin (PRL) receptors as well as down-regulation of breast cancer susceptibility gene product (brca1) through the cAMP-PKA/MAPK/Raf-ERK signalling pathways (De Petrocellis *et al.*, 1998; Melck *et al.*, 1999, 2000; Bifulco *et al.*, 2008) (Figure 1, left inset). Furthermore, it has been demonstrated that Δ^9-THC decreased the levels of Cdc2 (major cyclin dependent kinase controlling the entrance of cells in mitosis), thereby causing cell cycle arrest and subsequent apoptosis (Caffarel *et al.*, 2006) (Figure 1, left inset). Other signalling pathways could be involved or discovered in future studies.

In vivo studies demonstrate that cannabinoids reduce tumour growth and metastasis as well as cell proliferation and angiogenesis in mice injected with different breast cancer cell lines. For example, Δ^9-THC decreases tumour size as well as the number of tumour and lung metastases and inhibits both cell proliferation and angiogenesis in an engineered animal model of ErbB2 (tyrosine kinase receptor)-driven metastatic breast cancer (Caffarel *et al.*, 2010) (see Table 2 for more details). This inhibition of cell proliferation involves CB_2, but not CB_1 receptors (Caffarel *et al.*, 2010). Further evidence for a role for CB_2 receptors in these anti-cancer properties is based upon the ability of the CB_2 agonist JWH-133 to decrease size and number of tumours, reduce the number and size of lung metastases, inhibit cell proliferation and decrease angiogenesis in mice injected with different breast cancer cell lines (Qamri *et al.*, 2009; Caffarel *et al.*, 2010). These effects were mediated by CB_2, but not CB_1 receptors (Qamri *et al.*, 2009; Caffarel *et al.*, 2010). In CB-17 immunodeficient mice injected with MDA-MB-231 cells, the mixed CB_1/CB_2 agonist WIN55,212-2 also reduces tumour size, decreases the number and size of lung metastases, inhibits proliferation and reduces angiogenesis; these effects were mediated by CB_1 and CB_2 receptors (Qamri *et al.*, 2009) (Table 2). Furthermore, the phytocannabinoid cannabidiol (CBD) also reduces tumour growth (size) and decreases the number of lung metastases in mice injected with MDA-MB-231(Ligresti *et al.*, 2006) or 4T1 (McAllister *et al.*, 2010) breast cancer cell lines (see Table 2). Moreover, the anandamide analogue methanandamide also reduces the number and size of lung tumour nodules in mice injected with TSA-1 mammary carcinoma cell line through a CB_1 receptor mechanism (Grimaldi *et al.*, 2006) (Table 2). Strikingly, the CB_1 antagonist SR141716A, administered alone, has also been reported to decrease tumour size in mice injected with MDA-MB-231 cancer cells (Sarnataro *et al.*, 2006); more work is necessary to determine whether effects of SR141716A observed here can be attributed to direct activation of CB_1 receptors or to other receptor mechanisms (e.g. GPR55). However, conflicting data are reported in the literature in this regard because systemic administration of Δ^9-THC has been reported to increase the local tumour size and the number/size of metastasis in mice injected with 4T1 tumour cells into the rear foot-pads (McKallip *et al.*, 2005). These unusual findings are potentially explained by the fact that Δ^9-THC suppresses the anti-tumour immune response which involves CB_2, but not the CB_1 receptors (McKallip *et al.*, 2005). Moreover, SCID-NOD mice, which are devoid of anti-tumour immune responses, do not exhibit increases in tumour size or metastasis following Δ^9-THC administration

Table 2

Breast cancer *in vivo* studies

Mouse strain	Tumour induction (cell line)	Drugs	Dose and route	Tumour		Metastasis		Proliferation (pro)/apoptosis (apo)/angiogenesis (ang)	Mediated by		Reference
				Size	Number	Size	Number		CB_1	CB_2	
BALB/c	4T1 paw	Δ^9-THC	12.5–50 mg·kg^{-1} i.p.	↑ by Δ^9-THC	–	↑ by Δ^9-THC	↑ by Δ^9-THC	–	–	–	McKallip *et al.*, 2005
BALB/c	4T1 paw	Δ^9-THC	25–50 mg·kg^{-1} i.p.	↑ by Δ^9-THC	–	–	↑ by Δ^9-THC	–	–	–	McKallip *et al.*, 2005
SCID-NOD	4T1 paw	Δ^9-THC	25 mg·kg^{-1} i.p.	Δ^9-THC no Δ	–	–	Δ^9-THC no Δ	–	–	–	McKallip *et al.*, 2005
C57BL/6N	TSA-E1 paw	MET	0.5 mg·kg^{-1} i.p.	–	–	↓ by MET	↓ by MET	–	SR1	–	Grimaldi *et al.*, 2006
Athymic	MDA-MB-231 dorsal side	CBD	5–6.5 mg·kg^{-1} s.c.	↓ by CBD	–	–	–	–	–	–	Ligresti *et al.*, 2006
BalB/c	MDA-MB-231 paw	CBD	5–6.5 mg·kg^{-1} s.c.	–	–	–	↓ by CBD	–	–	–	Ligresti *et al.*, 2006
CD1	MDA-MB-231 flank	SR1	0.7 mg·kg^{-1} s.c.	↓ by SR1	–	–	–	–	–	–	Sarnataro *et al.*, 2006
CB-17 immuno	MDA-MB-231 flank	JWH-133	5 mg·kg^{-1} p.t.	↓ by JWH-133	–	–	–	JWH-133 ↓ pro, ↓ ang	–	SR2	Qamri *et al.*, 2009
CB-17 immuno	MDA-MB-231 flank	WIN-2	5 mg·kg^{-1} p.t.	↓ by WIN-2	–	–	–	WIN-2 ↓ pro, ↓ ang	AM251	SR2	Qamri *et al.*, 2009
CB-17 immuno	MDA-MB231-luc-D3H2LN lateral vein	JWH-133	5 mg·kg^{-1} i.p.	–	–	↓ by JWH-133	↓ by JWH-133	–	–	SR2	Qamri *et al.*, 2009
CB-17 immuno	MDA-MB231-luc-D3H2LN lateral vein	JWH-133, WIN-2	5 mg·kg^{-1} i.p.	–	–	↓ by WIN-2	↓ by WIN-2	–	AM251	SR2	Qamri *et al.*, 2009
PyMT	NA	JWH-133	5 mg·kg^{-1} i.p.	↓ by JWH-133	–	–	–	JWH-133 ↓ pro, ↓ ang	–	–	Qamri *et al.*, 2009
MMTV-neu	NA	Δ^9-THC	0.5 mg p.t.	↓ by Δ^9-THC	↓ by Δ^9-THC	–	↓ by Δ^9-THC	Δ^9-THC ↓ pro, ↑ apo, ↓ ang	Not SR1 pro	SR2 pro	Caffarel *et al.*, 2010
MMTV-neu	NA	JWH-133	0.05 mg p.t.	↓ by JWH-133	↓ by JWH-133	↓ by JWH-133	–	JWH-133 ↓ pro, ↑ apo, ↓ ang	Not SR1 pro	SR2 pro	Caffarel *et al.*, 2010
BalB/c	4T1 fourth major nipple	CBD	1–5 mg·kg^{-1} s.c.	↓ by CBD	–	↓ by CBD	↓ by CBD	–	–	–	McAllister *et al.*, 2010

↑, increase; ↓, decrease; –, not tested; ang, angiogenesis; apo, apoptosis; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CBD, cannabidiol; Δ^9-THC, deltat 9-tetrahydrocannabinol; immuno, immunodeficient; i.p., intraperitoneal; MET, methanandamide; MMTV-neu mice, genetically engineered mice of ErbB2 (tyrosine kinase receptor)-driven metastatic breast cancer; NA, not applicable; no Δ, no change; pro, proliferation; p.t., peritumoral; PyMT mice, transgenic mice developing mammary gland tumours; s.c., subcutaneous; SCID-NOD, devoid of anti-tumour immune response; SR1, SR141716A; SR2, SR144528; WIN-2, WIN55,212-2.

(McKallip *et al.*, 2005). None of these models of breast cancer is actually injecting tumour cells into the milk ducts or lobules of the breast which diminishes the translation relevance to human breast cancer and reinforces the need to develop new animal models that better reproduce the disease state.

Prostate cancer

Prostate cancer is the second most frequently diagnosed malignancy in men (Bray *et al.*, 2010; Ferlay *et al.*, 2010). Genetics, diet, medical exposure and viral infection are all factors implicated in occurrence or incidence of the disease (Djulbegovic *et al.*, 2010). Prostate cancer develops in the prostate, a gland in the male reproductive system. Most prostate cancers grow slowly although cases of aggressive prostate cancers are also observed. These cancer cells may metastasize from the prostate to other parts of the body, particularly to the bones and lymph nodes (Parkin *et al.*, 2001; Schroder *et al.*, 2009; Djulbegovic *et al.*, 2010).

Several studies have also evaluated the expression of cannabinoid receptors in different prostate cancer tissue/cell lines. Indeed, it was shown that high CB_1 receptor immunoreactivity score in prostate cancer tissue is associated with prostate cancer severity and outcome (Chung *et al.*, 2009). It was also demonstrated that CB_1 expression is up-regulated in prostate cancer tissue (Czifra *et al.*, 2009). Moreover, multiple prostate cancer cell lines (i.e. PC-3, DU-145, LNCaP, CWR22Rv1, CA-HPV-10) and human prostate cancer tissues express CB_1 receptors using RT-PCR, immunofluorescence, and Western blot (Ruiz *et al.*, 1999; Melck *et al.*, 2000; Sánchez *et al.*, 2003a; Nithipatikom *et al.*, 2004; Sarfaraz *et al.*, 2005; Chung *et al.*, 2009; Czifra *et al.*, 2009; Brown *et al.*, 2010). CB_2 receptors are also expressed in different prostate cancer cell lines (PC-3, DU-145, LNCaP, CWR22Rv1, CA-HPV-10) using RT-PCR, immunofluorescence and Western blot (Melck *et al.*, 2000; Sánchez *et al.*, 2003a; Nithipatikom *et al.*, 2004; Sarfaraz *et al.*, 2005; Brown *et al.*, 2010). Moreover, expression of FAAH is demonstrated in prostate cancer cell lines (PC-3, DU-145, LNCaP) and human prostate cancer tissue using Western blot, immunohistochemistry and RT-PCR (Ruiz-Llorente *et al.*, 2004; Endsley *et al.*, 2008; Takeda *et al.*, 2008; Brown *et al.*, 2010; Thors *et al.*, 2010; Wang *et al.*, 2008) (Table 3). Furthermore, the putative cannabinoid receptor GPR55 is also expressed in PC-3 and DU-145 prostate carcinoma cell lines using Western blot and RT-PCR (Piñeiro *et al.*, 2011). Thus, multiple cannabinoid receptor subtypes and endocannabinoid hydrolysing enzymes are localized to prostate tissue and synthetic cannabinoids, endocannabinoids and related compounds inhibit prostate cancer cell proliferation and produce apoptosis through CB_1 and/or CB_2 receptor mechanisms.

In vitro studies have extensively evaluated the ability of different endocannabinoids or cannabis-like compounds to inhibit prostate cancer cell proliferation and/or induce apoptosis in different prostate carcinoma (PC-3, DU-145, LNCaP, CWR22Rv1, CA-HPV-10) cell lines (Table 3). Δ^9-THC possesses anti-proliferative (Velasco *et al.*, 2001) and pro-apoptotic properties in prostate cancer cell lines (Ruiz *et al.*, 1999). The pro-apoptotic effects of Δ^9-THC are not mediated by CB_1 receptors, although a possible role for CB_2 receptors was not assessed (Ruiz *et al.*, 1999). Methanandamide has also been shown to inhibit prostate cancer cell proliferation (Melck *et al.*, 2000; Nithipatikom *et al.*, 2004; Olea-Herrero *et al.*, 2009a,b) and induce apoptosis (Olea-Herrero *et al.*, 2009a); these effects are mediated by CB_2, but not CB_1 receptors. Both Δ^9-THC and methanandamide induce cell proliferation at nanomolar concentrations (Velasco *et al.*, 2001; Sánchez *et al.*, 2003a,b), suggesting that the observed effects are likely to be physiologically relevant. The mixed cannabinoid agonist WIN55,212-2 also inhibits cell proliferation (Nithipatikom *et al.*, 2004; Sarfaraz *et al.*, 2005) and induces apoptosis (Sarfaraz *et al.*, 2005, 2006); these effects are mediated by both CB_1 and CB_2 receptors (Sarfaraz *et al.*, 2005). Furthermore, the CB_2-preferring agonist JWH-015 has anti-proliferative (Sánchez *et al.*, 2003b; Olea-Herrero *et al.*, 2009a) and pro-apoptotic properties (Olea-Herrero *et al.*, 2009a) and these effects are mediated by CB_2, but not CB_1 receptors (Olea-Herrero *et al.*, 2009a). HU-210 also inhibits cell proliferation (Melck *et al.*, 2000), although pharmacological specificity was not evaluated. Finally, synthetic endocannabinoids also exhibit anti-proliferative properties. In fact, AEA inhibits cell proliferation through CB_2, but not CB_1 receptors (Melck *et al.*, 2000; Mimeault *et al.*, 2003), whereas induction of apoptosis involves both CB_1 and CB_2 receptors (Mimeault *et al.*, 2003). Inhibition of endocannabinoid hydrolysis by methyl arachidonyl fluorophosphonate (Nithipatikom *et al.*, 2004), which targets FAAH and MGL, and by CAY10401 (Endsley *et al.*, 2008), which aims FAAH, suggests that elevation of endocannabinoids also inhibits cell proliferation. Inhibition of 2-AG hydrolysis by OTFP (3-octylthio-1,1,1-trifluoropropan-2-one), a compound containing a trifluoromethylketone moiety, also inhibited cell proliferation (Nithipatikom *et al.*, 2005; Endsley *et al.*, 2007) through a CB_1-dependent mechanism (Nithipatikom *et al.*, 2005). More work is necessary to demonstrate the specificity of OTFP for MGL in the model system. Phytocannabinoids including CBD, cannabigerol and cannabichromene, cannabidiol acid and THC acid (Ligresti *et al.*, 2006) as well as putative endocannabinoids such as arvanil (Melck *et al.*, 2000) and noladin ether (Nithipatikom *et al.*, 2004) all inhibit cell proliferation in different prostate cancer cell lines (Table 3). Anti-proliferative effects of arvanil are mediated by CB_1, but not CB_2 receptors (Melck *et al.*, 2000). Interestingly, palmitoylethanolamide (PEA) enhances the anti-proliferative effects of AEA, arvanil and HU-210 in a prostate cancer cell line (Di Marzo *et al.*, 2001), suggestive of an entourage effect. Omega-3 fatty-acid ethanolamides such as eicosapenta enoyl ethanolamide (EPEA) also exhibit anti-proliferative properties which involve both CB_1 and CB_2 receptors (Brown *et al.*, 2010).

In prostate cancer cells, cannabinoids, following receptor binding, inhibit cell proliferation and induce cell cycle arrest and apoptosis through cAMP-PKA/Raf-ERK signalling pathways. Indeed, treatment with anandamide produced an inhibition of epiderdmal growth factor (EGF)-induced proliferation via cell cycle arrest in prostate cancer cells and a down-regulation of EGF receptors levels (Mimeault *et al.*, 2003; Bifulco *et al.*, 2008) (Figure 1, right inset). Another study showed that treatment with WIN55,212-2 decreased androgen receptor (AR) expression and prostate specific antigen (PSA) levels in prostate cancer cells and also induced

Table 3

Prostate cancer *in vitro* and *ex vivo* studies

Human cell line/tissue	Expressing	Apoptosis	Proliferation	Apoptosis/proliferation mediated by CB_1	CB_2	Reference
PC-3	CB_1	↑ by Δ^9-THC	–	Not AM251	–	Ruiz *et al.*, 1999
DU-145	CB_1, low CB_2	–	↓ by AEA, 2-AG, MET, HU-210, arvanil	SR1 for AEA, arvanil	Not SR2 for AEA, arvanil	Melck *et al.*, 2000
DU-145	–	–	↓ by AEA, arvanil, HU-210 enhanced by PEA	–	–	Di Marzo *et al.*, 2001
PC-3	–	–	↓ by Δ^9-THC	Not SR1	–	Velasco *et al.*, 2001
PC-3	–	–	↑ by Δ^9-THC	SR1	–	Velasco *et al.*, 2001
PC-3	–	↑ by AEA	↓ by AEA	SR1 for apo	SR2 for apo; not SR2 for pro	Mimeault *et al.*, 2003
LNCaP DU-145	–	↑ by AEA	↓ by AEA	SR1 for pro	Not SR2 for pro	Mimeault *et al.*, 2003
PC-3	CB_1, CB_2	–	↑ by Δ^9-THC, MET	–	–	Sánchez *et al.*, 2003a
LNCaP	–	–	↑ by Δ^9-THC, MET	SR1 for MET	SR2 for MET	Sánchez *et al.*, 2003b
LNCaP	–	–	↓ by JWH-015	–	–	Sánchez *et al.*, 2003b
PC-3 DU-145	CB_1, CB_2	–	↓ by Noladin ether, WIN-2, MET	–	–	Nithipatikom *et al.*, 2004
PC-3 DU-145	CB_1, CB_2	–	↓ by endocannabinoid hydrolysis blockade (MAFP)	SR1	–	Nithipatikom *et al.*, 2004
LNCaP	CB_1, CB_2	–	–	–	–	Nithipatikom *et al.*, 2004
PC-3	FAAH	–	–	–	–	Ruiz-Llorente *et al.*, 2004
PC-3	–	–	↓ by 2-AG hydrolysis blockade (OTFP)	SR1	–	Nithipatikom *et al.*, 2005
DU-145	–	–	↓ by 2-AG hydrolysis blockade (OTFP)	–	–	Nithipatikom *et al.*, 2005
LNCaP	CB_1, CB_2	↑ by WIN-2	↓ by WIN-2	SR1	SR2	Sarfaraz *et al.*, 2005
DU-145 PC-3 CWR22Rv1 CA-HPV-10	CB_1, CB_2	–	–	–	–	Sarfaraz *et al.*, 2005
DU-145	–	CBD no Δ	↓ by CBD, CBG, CBC, CBD acid, THC acid	–	–	Ligresti *et al.*, 2006
LNCaP	–	↑ by WIN-2	–	–	–	Sarfaraz *et al.*, 2006
PC-3	–	–	↓ by 2-AG hydrolysis blockade (OTFP)	–	–	Endsley *et al.*, 2007
Prostate tumour	FAAH high	–	–	–	–	Endsley *et al.*, 2008
LNCaP	FAAH high	–	↓ by FAAH blockade (CAY10401)	–	–	Endsley *et al.*, 2008
DU-145	FAAH medium	–	–	–	–	Endsley *et al.*, 2008
PC-3	FAAH low	–	–	–	–	Endsley *et al.*, 2008
LNCaP PC-3 DU-145	FAAH	–	–	–	–	Wang *et al.*, 2008
Prostate tumour	CB_1 high in severe cancer	–	–	–	–	Chung *et al.*, 2009
Prostate tumour	CB_1 high	–	–	–	–	Czifra *et al.*, 2009
PC-3	–	↑ by MET, JWH-015	↓ by MET, JWH-015	Not SR1	SR2 apo	Olea-Herrero *et al.*, 2009a
LNCaP DU-145	–	↑ by MET, JWH-015	↓ by MET, JWH-015	–	–	Olea-Herrero *et al.*, 2009a
PC-3	–	↑ by MET	↓ by MET	Not SR1	SR2 partially	Olea-Herrero *et al.*, 2009b
LNCaP	CB_1, CB_2, FAAH	–	↓ by EPEA	–	–	Brown *et al.*, 2010
PC-3	CB_1, CB_2	–	↓ by EPEA	AM281	AM630	Brown *et al.*, 2010

↑, increase; ↓, decrease; –, not tested; 2-AG, 2-arachidonoyl glycerol; AEA, anandamide; apo, apoptosis; CAY10401, fatty-acid amide hydrolase inhibitor; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CBC, cannabichromene; CBD, cannabidiol; CBD acid, cannabidiol acid; CBG, cannabigerol; Δ^9-THC, delta-9 tetrahydrocannabinol; EPEA, eicospentaenoyl ethanolamide; FAAH, fatty-acid amide hydrolase; MAFP, methyl arachidonyl fluorophosphonate; MET, methanandamide; no Δ, no change; OTFP (3-octylthio-1,1,1-trifluoropropan-2-one; PEA, palmitoylethanolamide; pro, proliferation; SR1, SR141716A; SR2, SR144528; THC acid, tetrahydrocannabinol; WIN-2, WIN55,212-2.

Table 4

Bone cancer *in vitro* and *ex vivo* studies

Murine cell line\tissue	Tumour induction (cell line)	Expressing	Apoptosis	Bone loss	Reference
NCTC-2472	NA	CB_1, CB_2	WIN-2	–	Hald *et al.*, 2008
Femur	NCTC-2472	–	–	No Δ by WIN-2	Hald *et al.*, 2008
DRG	NCTC-2472	↑ by CB_2 ipsi; no Δ CB_1	–	–	Hald *et al.*, 2008
Spinal cord	NCTC-2472	No Δ CB_1, CB_2	–	–	Hald *et al.*, 2008
DRG	NCTC-2472	↑ by CB_1 ipsi	–	–	Khasabova *et al.*, 2008
DRG and plantar skin	NCTC-2472	↑ by FAAH activity	–	–	Khasabova *et al.*, 2008
Spinal cord	NCTC-2472	No Δ CB_1	–	–	Furuse *et al.*, 2009
Spinal cord and DRG	NCTC-2472	No Δ CB_2	–	–	Curto-Reyes *et al.*, 2010
Spinal cord and DRG	B16-F10	No Δ CB_2	–	–	Curto-Reyes *et al.*, 2010
Femur	NCTC-2472	–	–	↓ by AM1241	Lozano-Ondoua *et al.*, 2010

↑, increase; ↓, decrease; –, not tested; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; DRG, dorsal root ganglion; FAAH, fatty-acid amide hydrolase; ipsi, ipsilateral side; no Δ, no change; NA, not applicable; WIN-2, WIN55,212-2.

apoptosis (Sarfaraz *et al.*, 2005, 2006) (Figure 1, right inset). However, other signalling pathways may also be involved or implicated in the modulation of apoptosis, cell cycle arrest and/or proliferation by cannabinoids.

One *in vivo* model of prostate cancer has evaluated the ability of a cannabinoid to inhibit tumour growth. In this model, subcutaneous injection of PC-3 cells (prostate carcinoma cells) in the right flank of athymic nude male mice induced the development of tumours (Olea-Herrero *et al.*, 2009a). Interestingly, direct peritumoral administration of the CB_2 preferring agonist JWH-015 reduced tumour growth in the mice and this reduction of growth was inhibited by the CB_2 receptor antagonist SR144528 (Olea-Herrero *et al.*, 2009a). Injection of canine prostate carcinoma (ACE-1) cells into the mouse femur produces bone pain and bone remodelling, sprouting of calcitonin gene-related peptide (CGRP) and of sensory nerve fibres (Jimenez-Andrade *et al.*, 2010a), changes which impact processes implicated in bone metastasis observed following prostate cancer. The development of new animal models which better reproduce prostate cancer observed clinically may improve prostate cancer treatment; injection of prostate tumour cells into the flank limits translational relevance of the model and does not truly reproduce cancer originating in the prostate gland.

Bone cancer

Primary bone cancer identified as sarcomas arises in the cartilage. Chondrosarcoma and osteosarcoma are the most frequent primary bone cancers (Bovée *et al.*, 2010). Secondary bone cancer is more frequent as it is associated with the wide spread of other cancers through bone metastases from lung or other solid tumours (Mercadante, 1997; Portenoy *et al.*, 1999).

In mice injected with NCTC-2472 sarcoma cell lines, CB_1 (Khasabova *et al.*, 2008) or CB_2 (Hald *et al.*, 2008) receptors are up-regulated in DRG ipsilateral to cancer bearing limb. However, no change in the expression of CB_1 (Hald *et al.*, 2008; Furuse *et al.*, 2009) or CB_2 (Hald *et al.*, 2008; Curto-Reyes *et al.*, 2010) receptors was observed in the spinal cord, suggesting that the observed up-regulation was restricted to the periphery. Another study failed to observe up-regulation of CB_2 in DRG (Curto-Reyes *et al.*, 2010). Furthermore, FAAH activity is also increased in DRG and plantar skin of mice injected with NCTC-2472 sarcoma cells (Khasabova *et al.*, 2008).

In vitro studies demonstrate that multiple cannabinoid or cannabis-derived compounds induce apoptosis and/or reduce bone resorption in different bone sarcoma (i.e. NCTC-2472, B16-F10) cell lines (Table 4). WIN55,212-2 induces apoptosis in the NCTC-2472 sarcoma cell line (Hald *et al.*, 2008). Furthermore, the CB_2 agonist AM1241 produced a reduction in bone loss in the femur of mice injected with NCTC-2472 sarcoma cell line (Lozano-Ondoua *et al.*, 2010). Thus, regulatory changes in the endocannabinoid system (in DRG and plantar skin) are observed in models of bone cancer pain and activation of cannabinoid CB_1 or CB_2 receptors produces antinociception and apoptosis. Activation of CB_2 receptors additionally reduces bone loss in tumour-treated mice, suggesting that many aspects of cannabinoid pharmacology may promote anti-cancer effects.

In vivo bone cancer models differ from breast and prostate cancer models in that they more readily lend themselves to direct assessments of therapeutic effects of cannabinoids such as anti-nociception (Jimenez-Andrade *et al.*, 2010b). These studies have evaluated the efficacies of cannabinoids in suppressing tumour-evoked hyperalgesia and/or reductions of tumour growth and metastasis. Injection of different cancer (fibrosarcoma NCTC-2472 or melanoma B16-F10) cells into the calcaneous, tibial or femur bones of mice produces bone tumours (Table 5). Systemic injection of CP55 940 produced anti-nociceptive properties in the tail flick test and suppressed mechanical hyperalgesia (to von Frey stimulation) in this model (Hamamoto *et al.*, 2007). These anti-nociceptive effects were mediated by CB_1, but not CB_2 receptors

Table 5

Bone cancer *in vivo* studies

Mouse strain	Tumour induction (cell line)	Drugs	Dose and route	Anti-nociception	Mechanical anti-allodynia	Catalepsy	Mediated by CB_1	Mediated by CB_2	Reference
C3H/HeNCr	NCTC-2472 calcaneous	CP55,940	0.1–3 mg·kg^{-1} i.p.	CP55,940 TF	CP55,940 VF	CP55,940 BT	SR1 TF, VF, BT	Not SR2 TF, VF, BT	Hamamoto *et al.*, 2007
C3H/HeN	NCTC-2472 femur	WIN-2	0.5–5 mg·kg^{-1} s.c.	WIN-2 WB, OF	WIN-2 VF	–	–	–	Hald *et al.*, 2008
C3H/HeN	NCTC-2472 calcaneous	AEA	1 μg i.pl.	–	AEA VF	–	AM281 VF	Not AM630 VF	Khasabova *et al.*, 2008
C3H/HeN	NCTC-2472 calcaneous	URB597	9 μg i.pl.	–	URB597 VF	–	AM281 VF	Not AM630 VF	Khasabova *et al.*, 2008
C3H/He	NCTC-2472 calcaneous	WIN-2	1.5–10 μg i.pl.	–	WIN-2 VF	No WIN-2	AM251 VF	AM630 VF	Potenzieri *et al.*, 2008
C57BL/6J	NCTC-2472 femur	ACEA	1 nmol i.t.	ACEA SF, LU, WB	–	No ACEA	AM251 SF, LU, WB	Not AM630 SF, LU, WB	Furuse *et al.*, 2009
C57BL/6J	NCTC-2472 femur	URB597	0.3–3 nmol i.t.	No SF, LU, WB	–	–	–	–	Furuse *et al.*, 2009
C57BL/6J	NCTC-2472 femur	URB602	0.17–1.7 nmol i.t.	No SF, LU, WB	–	–	–	–	Furuse *et al.*, 2009
C3H/He	NCTC 2472 tibia	AM1241	1–10 mg·kg^{-1} i.p.	AM1241 HP	AM1241 VF	–	Not AM251 HP, VF	SR2 HP, VF	Curto-Reyes *et al.*, 2010
C3H/He	NCTC 2472 tibia	AM1241	0.1–1 μg i.t.	AM1241 HP	AM1241 VF	–	–	SR2 HP, VF	Curto-Reyes *et al.*, 2010
C57BL/6	B16-F10 tibia	AM1241	1–10 mg·kg^{-1} i.p.	AM1241 HP	AM1241 VF	–	Not AM251 HP, VF	SR2 HP, VF	Curto-Reyes *et al.*, 2010
C3H/HeJ	NCTC-2472 femur	AM1241	6 mg·kg^{-1} i.p.	AM1241 SF, LU	AM1241 VF	–	–	SR2 SF, LU, VF	Lozano-Ondoua *et al.*, 2010

↑, increase; ↓, decrease; –, not tested; AEA, anandamide; ACEA, arachidonyl-2′-chloroethylamide; BT, bar test; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; HP, hot plate; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; LU, limb use; no Δ, no change; OF, open field; s.c., subcutaneous; SF, spontaneous flinches; SR1, SR141716A; SR2 SR144528; TF, tail flick; VF, von Frey; WIN-2, WIN55,212-2; WB, weight bearing.

(Hamamoto *et al.*, 2007) (Table 5). Effects of subcutaneously administered WIN55,212-2 on weight bearing and mechanical hyperalgesia were consistent with cannabinoid receptor-mediated anti-nociception (Hald *et al.*, 2008). WIN55,212-2 also attenuates tumour-evoked mechanical hyperalgesia following local (intraplantar) administration through activation of CB_1 and CB_2 receptors (Potenzieri *et al.*, 2008). Endocannabinoids and modulators of the endocannabinoid system also attenuate tumour-evoked pain. Indeed, intraplantar administration of AEA reduces mechanical hyperalgesia and URB597, a potent inhibitor of FAAH, increases AEA levels and decreases hyperalgesia in a model of calcaneous bone cancer pain (Khasabova *et al.*, 2008). These effects of AEA and URB597 are mediated by CB_1, but not CB_2 receptors (Khasabova *et al.*, 2008). However, intrathecal administration of either FAAH (URB597) or MGL (URB602) inhibitors failed to produce anti-nociception when tested for spontaneous flinches, limb use and weight bearing (Furuse *et al.*, 2009). Moreover, the CB_1 agonist arachidonoyl-2-chloroethylamide (ACEA) produces anti–nociceptive properties following intrathecal administration in this model; ACEA suppressed spontaneous flinches and increased limb use and weight bearing through CB_1, but not CB_2 receptor mechanisms (Furuse *et al.*, 2009) (Table 5). Furthermore, systemic and intrathecal administration of the CB_2 agonist AM1241 produces anti-nociception measured with multiple dependent measures (i.e. flinches, limb use, hot plate, von Frey) (Curto-Reyes *et al.*, 2010; Lozano-Ondoua *et al.*, 2010). These anti-nociceptive effects, assessed with multiple different testing methods, are all mediated by CB_2, but not CB_1 receptors (Curto-Reyes *et al.*, 2010; Lozano-Ondoua *et al.*, 2010). Animal models of bone cancer closely reproduce human bone cancer because tumour cells are injected directly into the bone, where the cancer originates in humans. New techniques for injection of mammary rat metastasis tumour cells in the femur have also recently been developed in rats (Doré-Savard *et al.*, 2010).

Cannabinoids in clinical cancer studies

Cannabinoids have been evaluated in cancer patients for their anti-emetic, anti-nociceptive and orexigenic properties. Numerous studies have been published documenting the anti-emetic properties of cannabis-like compounds (Davis, 2008; Navari, 2009; Parker *et al.*, 2011 for reviews). Fewer clinical trials have evaluated pain relief following cannabinoid administration in cancer patients. Indeed, the role of cannabinoids in relieving pain associated with cancer has been evaluated in only five clinical studies. Four of these studies were performed more than 32 years ago (Noyes *et al.*, 1975a,b; Jochimsen *et al.*, 1978; Staquet *et al.*, 1978; Johnson *et al.*, 2010). In the initial study, oral THC, at doses of 15 and 20 mg, produced analgesic effects in patients experiencing cancer pain (Noyes *et al.*, 1975a). The same study showed that oral administration of single lower dose of Δ^9-THC (10 mg) to patients with cancer pain was well-tolerated and produced a mild analgesic effect, whereas higher doses of Δ^9-THC (20 mg) also produced adverse side effects (Noyes *et al.*, 1975b). Another study, using a nitrogen analogue of Δ^9-THC, showed that pain relief was superior to placebo in patients with cancer (Staquet *et al.*, 1978). Another group, also using a nitrogen analogue of Δ^9-THC, showed that the cannabinoid (2 or 4 mg) was not effective as an analgesic, compared with placebo and even appeared to augment pain perception in patients with chronic pain due to malignancies (Jochimsen *et al.*, 1978). A more recent study demonstrated that the administration of Sativex (Δ^9-THC: CBD in a 1:1 ratio) reduced pain scores when compared with placebo, whereas effects of Δ^9-THC administration alone on pain did not reach significance (Johnson *et al.*, 2010).

The present studies suggest that additional clinical trials evaluating the therapeutic efficacy of cannabinoids in cancer pain are warranted, particularly in light of other aspects of cannabinoid receptor pharmacology that hold considerable therapeutic potential (i.e. anti-emetic and anti-tumour properties).

Conclusion and limitations

The available literature suggests that the endocannabinoid system may be targeted to suppress the evolution and progression of breast, prostate and bone cancer as well as the accompanying pain syndromes. Although this review focuses on these three types of cancer, activation of the endocannabinoid signalling system produces anti-cancer effects in other types of cancer including skin, brain (gliomas) and lung (Velasco *et al.*, 2007; Bíró *et al.*, 2009; Pacher and Mechoulam, 2011 for reviews). Interestingly, cannabis trials in population-based studies failed to show any evidence for increased risk of respiratory symptoms/chronic obstructive pulmonary disease (Tan *et al.*, 2009; Hancox *et al.*, 2010) or lung cancer (Tashkin, 2005) associated with smoking cannabis. Moreover, synthetic cannabinoids and the endocannabinoid system play a role in inhibiting cancer cell proliferation and angiogenesis, reducing tumour growth and metastases and inducing apoptosis in all three types of cancers reviewed here. These observations raise the possibility that a dysregulation of the endocannabinoid system may promote cancer, by fostering physiological conditions that allow cancer cells to proliferate, migrate and grow. These observations also raise the exciting possibility that enhancing cannabinoid tone through cannabinoid-based pharmacotherapies may attenuate these harmful processes to produce anti-cancer effects in humans. However, the basic research findings are far from being completely understood and further research is warranted to better understand the complexity of dynamic changes in the endocannabinoid system in cancer. One of the reasons for this complexity is likely attributable to the highly interactive nature of lipid signalling pathways which recruit different signalling pathways and mechanisms of action. Indeed, endocannabinoids are known to interact with the cyclooxgenase enzyme, inhibit the transcription of genes implicated in metastasis processes, induce cell cycle arrest, activate the formation of reactive oxygen species and ensure the integrity of raft/caveolae needed for anti-proliferative properties. However, other mechanisms are also likely to be involved and interact with the endocannabinoid system in ways that are yet to be discovered.

Many *in vitro* and *in vivo* studies have shown that cannabinoids are efficacious in reducing cancer progression (i.e.

inhibition of tumour growth and metastases as well as induction of apoptosis and other anti-cancer properties) in breast, prostate and bone cancer. However, further research is needed because the complexity of the effects of cannabinoids and their interaction with other mechanisms and signalling pathways remain to be elucidated. The need for further study is particularly crucial in the case of prostate cancer; only one study, performed in mice, has evaluated *in vivo* effects of a cannabinoid (JWH-015) on tumour growth. The paucity of *in vivo* preclinical and clinical data is striking given the large number of compounds that have been tested *in vitro* in different types of prostate cancer cell lines.

Despite the need for further *in vitro* and *in vivo* studies, the literature is nearly unanimous in suggesting that cannabinoids and endocannabinoids reduce the progression of cancer in both *in vivo* preclinical and *in vitro* model systems. The need for additional clinical trials of cannabinoid therapeutic efficacy in cancer appears beyond doubt; only few studies have evaluated the effects of cannabinoid in alleviating cancer pain, in contrast to the extensive literature supporting efficacy of cannabinoids as anti-emetics. Furthermore, future research needs to explore the therapeutic potential of multimodal analgesic strategies that combine cannabinoids with other commonly used medications (opioids) or employ multiple phytocannabinoids in combination. The use of different pharmacotherapies in combination may increase the likelihood of synergistic interactions between compounds with multiple distinct mechanisms of action; such combinations may produce a more beneficial therapeutic ratio in cancer patients compared with conventional analgesics, resulting in improved pain relief and anti-cancer effects with fewer adverse side effects. Moreover, because cannabinoids attenuate neuropathy produced by cancer chemotherapy through CB_1 and CB_2-dependent mechanisms (Rahn and Hohmann, 2009 for review), the possibility remains that cannabinoids in combination with chemotherapy may enhance both anti-tumour actions of chemotherapy and attenuate unwanted iatrogenic side effects (e.g. emesis, neuropathy). Further basic research on cannabinoid anti-cancer properties as well as clinical trials evaluating cannabinoid efficacy in cancer are required before cannabinoid use can be established and accepted as effective adjuncts to cancer therapy.

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