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Cannabinoid pharmacology in cancer research: A new hope for cancer patients?

Cannabinoid pharmacology in cancer research

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Abstract

Cannabinoids have been used for many centuries to ease pain and in the past decade, the endocannabinoid system has been implicated in a number of pathophysiological conditions, such as mood and anxiety disorders, movement disorders such as Parkinson's and Huntington's disease, neuropathic pain, multiple sclerosis, spinal cord injury, atherosclerosis, myocardial infarction, stroke, hypertension, glaucoma, obesity, and osteoporosis. Several studies have demonstrated that cannabinoids also have anti-cancer activity and as cannabinoids are usually well tolerated and do not produce the typical toxic effects of conventional chemotherapies, there is considerable merit in the development of cannabinoids as potential anticancer therapies. Whilst the presence of psychoactive effects of cannabinoids could prevent any progress in this field, recent studies have shown the value of the non-psychoactive components of cannabinoids in activating apoptotic pathways, inducing anti-proliferative and anti-angiogenic effects. The aforementioned effects are suggested to be through pathways such as ERK, Akt, mitogen-activated protein kinase (MAPK) pathways, phosphoinositide 3-kinase (PI3K) pathways and hypoxia inducible factor 1 (HIF1), all of which are important contributors to the hallmarks of cancer. Many important questions still remain unanswered or are poorly addressed thus necessitating further research at basic pre-clinical and clinical levels. In this review, we address these issues with a view to identifying the key challenges that future research needs to address.

Keywords: Cannabinoids, cancer, cannabinoid receptors

Abbreviations:

ABCC1, ATP-binding cassette (ABC) transporter

AC, adenylyl cyclase

AEA, anandamide

AKt, protein kinase B

AM251, a CB₁ receptor antagonist

AM630, a CB₂ receptor antagonist

AMPK, 5'-adenosine monophosphate-activated protein kinase

ATF-4, activating transcription factor-4

AR, androgen receptors

CBD, cannabidiol

CB₁IR, CB₁ receptor immunoreactivity

Cdk, cyclin-dependant kinase

Chk 1, cell cycle checkpoint

COX2, cyclooxygenase-2

CXCR4, chemokine receptor 4

CXCL12, a chemokine protein encoded by the CXCL12 gene

Δ^9 -THC, Δ^9 -tetrahydrocannabinol

EGFR, epidermal growth factor receptor

ER, Oestrogen

ERK, extracellular signal-regulated kinase

FAAH, fatty acid amide hydrolase

FAK, focal adhesion kinase

GBM, glioblastoma multiform

Gi/o, a subunit of G protein

GTP γ S, guanosine 5'-O-[gamma-thio] triphosphate

HER2, human epidermal growth factor receptor 2

HIF- α , hypoxia-inducible factor

HU-210, highly potent cannabinoid receptor agonist, 96aR0-trans-3-91, 1-Dimethylheptyl)-

6a, 7, 10, 10a-tetrahydro-1-hydroxy-6, 6-dimethyl-6H-dibenzo [b,d]pyran-9-methanol

ICAM, intracellular adhesion molecule

JWH-015, a selective CB₂ agonist, 2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone

JWH-133, a potent selective CB₂ agonist, (6aR, 10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-

Tetrahydro-6, 6, 9-trimethyl-6H- dibenzo [b,d]pyran MAPK, mitogen activated protein kinase

LAK, lymphokine-activated killer

LPI, lysophosphatidylinositol

MAGL, monoglycerol lipase

MMP-2, matrix metalloproteinase 2

MMP-9, matrix metalloproteinase 9

MRP1, Multidrug resistance-related protein 1

mTOR, mammalian target of rapamycin

NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells

NGF, nerve growth factor

PD98059, p42/44 inhibitor, 2-(2-Amino-3-methoxyphenyl)-4-h-1-benzopyran-4-one

PGE₂, prostaglandin E-2

PI3K, phosphoinositide 3-kinase

PKA, protein kinase A

PCNA, proliferating cell nuclear antigen

PPARs, peroxisome proliferator-activated receptors

PR, progesterone

PRLr, prolactin receptor

PSA, prostate specific antigen

PyMT, polyoma middle T oncoprotein

RAF-1, a proto-oncogene, serine/threonine kinase

ROS, reactive oxygen species

RXR α , retinoid X receptor

SB203580, a p38/MAPK inhibitor, 4-[5-(4-Fluorophenyl)-2-[4-(methylsulphonyl) phenyl]-1H-

imidazol-4-yl] pyridine

SC58236, COX-2-specific inhibitor, 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide

siRNA, small interfering RNA

SR141716, Rimonabant, a selective CB₁ receptor antagonist or an inverse agonist

Src gene, a family of proto-oncogenic tyrosine kinases

Th1, a type of T helper cells

Th2, a type of T helper cells

TIMP-1, tissue inhibitor of matrix metalloproteinases-1

TrK A, tropomyosin receptor kinase A

TRPM8, transient receptor potential channels of melastatin-type 8

TRPVA1, transient receptor potential A1

TRPV1, transient receptor potential vanilloid 1

TRB3, tribbles homolog that inhibits Akt/PKB activation

2-AG, 2-arachidonoyl glycerol

VEGF, vascular endothelial growth factor

WIN 55,212-2, a CB₁ and CB₂ receptor agonist [(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanoneesylate]

1. Introduction

It is known that cannabinoids, the active components of *Cannabis sativa*, act by mimicking the endogenous substances (the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG)) by activating specific cell-surface cannabinoid receptors (Devane et al., 1992). Currently, the cannabinoid receptor ligands are generally divided into three main categories known as phytocannabinoids, endogenous cannabinoids and synthetic cannabinoids (figure 1). After the

clarification of the chemical structure of (-)- Δ^9 -tetrahydrocannabinol (Δ^9 -THC) which is the primary psychoactive component of the cannabis plant (Gaoni, 1964a; Gaoni, 1964b), other chemically related terpenophenolic compounds were identified in *Cannabis sativa*, including cannabichromene (CBC) (Gaoni, 1966) and cannabigerol (CBG) (Gaoni, 1964c). Although the pharmacology of most of the cannabinoids is unknown, Δ^9 -THC is the most widely studied owing to its high potency and abundance in cannabis (Pertwee et al., 2010). Among the herbal cannabinoids, other relevant plant-derived cannabinoids include Δ^8 -THC, which is almost as active as Δ^9 -THC but less abundant and cannabinol (CBN), which is produced in large amounts but is a weak cannabomimetic agent. Cannabidiol (CBD), CBG and CBC are devoid of psychoactive potential. The chemical structures of some cannabinoids are shown in figure 1.

So far, two cannabinoid-specific receptors CB_1 and CB_2 have been cloned and characterized from mammalian tissues (Howlett et al., 2002). Mouse CB_1 receptor and CB_2 receptor share 66% overall homology and 78% in the transmembrane region (Shire et al., 1996). Human CB_1 receptor and CB_2 receptor share an overall homology of 44%, and 68% in the transmembrane region respectively (Munro et al., 1993). Homology (96%) has been reported between human and mouse CB_1 receptor (Chakrabarti et al., 1995), whilst human and mouse CB_2 receptors share 82% homology (Shire et al., 1996). Many central and peripheral effects have been associated with the activation of CB_1 -receptors (Matsuda et al., 1990; Munro et al., 1993; Pertwee, 2006; Pertwee et al., 2010). The CB_2 receptor, originally thought of as being exclusively present in the immune system, is highly expressed in B and T lymphocytes, macrophages and in tissues such as the spleen, tonsils and lymph nodes (Herkenham et al., 1991; Howlett et al., 2002; Porter et al., 2001; Pertwee et al., 2010). Recently CB_2 receptors have been shown to be also located in the brain stem (Van Sickle et al., 2005). Further studies using CB_1 knockout mice demonstrated that CB_1 receptors are involved in a variety of different behavioural disorders such as depression, anxiety, feeding and cognition as well as pain at the peripheral, spinal and supraspinal levels (Valverde et al., 2005). Such studies

using CB₁ knockout mice also revealed the interactions between different systems such as opioids, gamma aminobutyric acid (GABA) and cholecystinin (CCK) via CB₁ receptors (Valverde et al., 2005). CB₂ knockout mice have also been developed and revealed/confirmed the involvement of CB₂ receptors in a variety of different systems such as immune system, inflammation, apoptosis, chemotaxis, bone loss, liver disorder, pain and atherosclerosis (Buckley, 2008).

Both CB₁ and CB₂ receptors are metabotropic and belong to the G-protein coupled receptor family (Howlett et al., 2002). Activation of CB₁ and CB₂ receptors stimulates cellular signalling via alpha subunit of G protein (Gi/o), leading to inhibition of adenylate cyclase and the subsequent activation of many other pathways such as mitogen-activated protein kinase (MAPK) pathways, phosphoinositide 3-kinase (PI3K) pathways, modulation of ion channels (through CB₁ receptors), protein kinase B (Akt), ceramide signalling pathways in tumour cells and modulation of cyclooxygenase-2 (COX-2) signalling pathway (Demuth et al., 2006; Galve-Roperh et al., 2000; Glass et al., 1999; Guzman et al., 2001; Qamri et al., 2009).

There is also pharmacological evidence that non-CB₁ and non-CB₂ receptors mediate the actions of cannabinoids located in the brain (Breivogel et al., 2001; Di Marzo et al., 2000). The hypothesis that putative CB₃ or non-CB₁/CB₂ receptor exist is supported by the fact that some of the anandamide (AEA)-mediated effects were neither inhibited by selective CB antagonists nor fully abolished in knockout mice lacking CB₁ receptors (De Petrocellis et al., 2010). Recent advances suggest, at least for AEA, that the transient receptor potential vanilloid 1 receptor (TRPV1) channel may be considered as the “third” receptor involved in endocannabinoid signaling (Di Marzo et al., 2001; Ross, 2003). For example, it has been shown that the endocannabinoids exert their apoptotic effect by binding to TRPV1, a non-selective cation channel targeted by capsaicin, the active component of hot chilli peppers (Smart & Jerman, 2000). However, the precise role of this receptor in cannabinoid signalling is still unclear and this uncertainty extends into the cancer field where its potential role in cancer biology (proliferation and migration of cancer cells) and cancer

pharmacology (resistance to chemotherapeutic agents) needs further investigation (Lehen'kyi et al., 2011; Liberati et al., 2013). Evidence also exists supporting a role for peroxisome proliferator-activated receptors (PPARs) in the actions of cannabinoids (Sun et al., 2007). More recent studies have provided evidence for the interaction of cannabinoids with the orphan receptors such as G protein receptor 55 (GPR55) (Andradas et al., 2011; Pineiro et al., 2011). Thus in addition to CB₁ and CB₂ receptors other targets might be involved in mediating an effect to cannabinoids and endocannabinoids.

The potential of cannabinoids to alleviate pain has been recognized for many centuries. The antinociceptive actions are mediated via both the CB₁ and CB₂ receptors (Pacher et al., 2006). This does not negate a role for other receptors such as TRPV1, transient receptor potential cation channel A1 (TRPA1), orphan GPCR (i.e. GPR55) or PPAR- γ (Maione et al., 2006; Maione et al., 2013; Perez-Gomez et al., 2013; Moreno et al., 2014). For a long time, the development of cannabinoids as anticancer agents has been restricted to two therapeutic avenues (antiemetic and analgesic). They have therefore been evaluated in terms of palliative care as cannabinoids can play an important role in the relief of pain, nausea, vomiting, and stimulation of appetite in cancer patients. However, the involvement of CB receptors in pain and their use in the palliative care in cancer patients are not the focus of this review. In the present review, the aim is to focus on the anti-tumour effects of cannabinoids, identify potential mechanisms by which cannabinoids induce anti-tumour effects and discuss the potential and challenges for the future development of this class of compound.

2. Anti-tumour effects of cannabinoids

Whilst cannabinoids exert palliative effects in cancer patients by preventing nausea, vomiting and pain and by stimulating appetite, they have also been shown to inhibit the growth of tumour cells in culture and animal models by modulating key cell-signalling pathways. In 1975, Munson and collaborators were the first to report the anti-proliferative properties of cannabis compounds (Munson et al., 1975). They showed that Δ^9 -THC inhibits lung-adenocarcinoma cell

growth *in vitro* and after oral administration in mice. It was not until the late 1990s however that further studies on the anti-cancer effects of cannabinoids were carried out. Several plant-derived (for example, Δ^9 -THC and CBD), synthetic (for example, WIN-55, 212-2 and HU-210) and endogenous cannabinoids (for example, AEA and 2-AG) are now known to exert anti-proliferative actions on a wide range of tumour cells *in vitro* (Guzman et al., 2002). The involvement of CB₁ and/or CB₂ receptors in the anti-tumour effects of cannabinoids has been shown by various biochemical and pharmacological approaches, in particular by determining cannabinoid-receptor expression and by using selective cannabinoid-receptor agonists and antagonists (Guzman et al., 2003; Sarfaraz et al., 2008; Pisanti et al., 2013 ;Velasco et al., 2012). Such studies have shown that cannabinoids can prevent proliferation, metastasis, angiogenesis and exert pro-apoptotic effects in a variety of cancer cell types such as lung, breast, prostate, skin, intestine, glioma, lymphoma, pancreas and uterus (Blazquez et al., 2006; Carracedo et al., 2006b; Casanova et al., 2003; Cianchi et al., 2008; Galve-Roperh et al., 2000; Guzman, 2003; Pacher et al., 2006; Sanchez et al., 2001a; Sanchez et al., 2001b). Silencing CB₂ receptors with specific small interfering RNA (siRNA) in **prostate cancer cells** (PC-3) revealed the involvement of CB₂ receptors in the growth inhibition of prostate cancer cells (Olea-Herrero et al., 2009) via stimulation of autophagy. Cannabinoid-induced AMPK activation of autophagy was also confirmed *in vivo* (Vara et al., 2011). The over expression of cannabinoid receptors and elevated endocannabinoid levels have also been reported in a variety of different cancer types such as prostate, skin, hepatocellular carcinoma, colon, endometrial sarcoma, glioblastoma multiforme (GBM), meningioma and pituitary adenoma (see table 1) (Blazquez et al., 2006; Xu et al., 2006; Pisanti et al., 2013). CB₁ receptors are also up-regulated in Hodgkin lymphoma cells and also in chemically induced hepatocarcinoma (Benz et al., 2013; Mukhopadhyay et al., 2015). Also the expression of CB₁ and CB₂ receptors was found to increase in mantel cell lymphoma and fatty acid amide hydrolase (FAAH) expression was reduced when compared to non-malignant B-cells (Islam et al., 2003; Ek et al., 2002; Wasik et al., 2014). It has also been shown in both the mouse model of metastatic melanoma and in humans that the

circulating endocannabinoid levels have been associated with an increase in disease progression (Sailler et al., 2014). Indeed many reports have shown that an increase in the level of endocannabinoids and their receptors correlates with tumour aggressiveness (Malfitano et al., 2011) and that cannabinoids can inhibit the growth of xenograft tumours (Blazquez et al., 2006; Carracedo et al., 2006b; Sanchez et al., 2001a).

In neoplastic cells cannabinoids have also been shown to inhibit angiogenesis and directly initiate apoptosis or cell cycle arrest (Blazquez et al., 2006; Carracedo et al., 2006b; Sanchez et al., 2001a). Other studies have shown the ability of cannabinoids to affect cellular signalling pathway/s essential for cell survival and growth (Bifulco et al., 2008; Kogan, 2005). Some studies have also demonstrated and suggested the involvement of autophagy in the mechanism of cannabinoid-induced cytotoxicity (Armstrong et al. 2015; Vara et al., 2011; McAllister et al., 2015). Cannabinoids have also been reported to inhibit nerve growth factor (NGF)-induced proliferation of PC-3 cells (Melck et al., 2000) through interaction with CB₁ receptors and synthetic endocannabinoid-vanilloid hybrids via stimulation of TRPV1 channels. **However, other studies demonstrate that cannabinoid-induced anti-tumour activity was only marginally dependent upon the interaction between cannabinoid and TRPV1 receptors (Massi et al., 2004; Torres et al., 2011; Vaccani et al., 2005).** On the other hand, cannabinoids have also been shown to actively induce apoptosis in these cells via a CB₁ receptor-independent mechanism as the Δ^9 -THC-induced apoptosis was not reversed by the CB₁ receptor antagonist, SR141716 (Ruiz et al., 1999).

The effects of cannabinoids in modulating cell cycle and signalling pathways are diverse (Figure 2) and may depend on the type of tumour cells. Whilst there is evidence showing that some cannabinoids induce anti-proliferative effect on tumour cells *in vitro* (Hart et al., 2004; White et al., 1976; Velasco et al., 2015), there is evidence to suggest that the effects may depend upon the disease context with differential effects seen in different tumour types. The subsequent sections focus specifically on the evidence of cannabinoid induced anti-tumour effects in specific cancer

types with the aim of exploring the mechanism of action of non-psychoactive components of cannabinoids in a disease specific context.

3. Cannabinoids and breast cancer

The first report on the antineoplastic property of cannabinoids in breast cancer are in the late 1990s, when it was shown that pre-treatment with the endocannabinoid anandamide inhibited prolactin- and nerve growth factor-induced proliferation of two hormone-sensitive, oestrogen and progesterone (ER^+/PR^+) **breast cancer cell lines** (EFM-19 and MCF-7 cell lines). In this case, treatment reduced the levels of prolactin receptor (PRLr) and nerve growth factor receptors via CB_1 receptor activation. Thus, the inhibition of adenylyl cyclase activity that in turn induced prolonged activation/stimulation of the RAF1-MEK-ERK cascade, leads to a down-regulation of the PRL receptor and levels of TrkA NGF receptors (De Petrocellis et al., 1998; Melck et al., 2000; Melck et al., 1999). Of interest, other studies showed that in both MCF-7 cells and **tamoxifen-resistant MCF-7** (TAMR-MCF-7) tumour cells, anti-angiogenesis effects exerted by novel synthetic hexahydrocannabinol analogues was through the suppression of VEGF (Thapa et al., 2011). It should be noted that both MCF-7 and TAMR-MCF-7 cells have shown a strong association between enhanced VEGF production and more aggressive phenotype (Kim et al., 2008; Kim et al., 2009). The anti-angiogenesis activity afforded by the novel synthetic cannabinoids were shown to be independent of CB_1 and CB_2 receptor activity and through an inhibition of NF- κ B transcriptional activity which in turn plays an important role in VEGF regulation and angiogenesis (Thapa et al., 2011). Further studies demonstrated that the proliferation of EVSA-T, **a hormone-sensitive (ER^-/PR^+) breast cancer cell line**, was also inhibited by TCH via CB_2 receptor activation. This caused the activation of the transcription factor JunD, the up-regulation of gene expression and subsequent translocation of protein to the nuclear compartment (Caffarel et al., 2008). Thus, it seems that cannabinoids do activate many cell-specific pathways, however not all pathways are simultaneously stimulated (Figure 3). In addition to their anti-mitogenic property, cannabinoids have been also

shown to play a role in hormone-sensitive breast cancer cell migration and invasion. Specifically, CB₂ has recently been found to modulate breast tumour growth and metastasis by inhibiting signalling of the chemokine receptor CXCR4 and its ligand CXCL12 in both in MCF7 overexpressing CXCR4 and NT2.5 injected immune-competent syngenic FVB mice (Nasser et al., 2011). However, other studies showed that Δ^9 -THC enhanced breast cancer growth and metastasis although specifically in cells expressing low levels of cannabinoid receptors (i.e. mouse mammary carcinoma 4T1 cells) by suppressing the antitumor immune response (McKallip et al., 2005). Such studies showed that exposure to Δ^9 -THC led to an increase in the level of cytokines such as IL-4 and IL-10, suggesting that Δ^9 -THC exposure may specifically suppress the cell-mediated Th1 response by enhancing Th2-associated cytokines (McKallip et al., 2005). Among the hormone-sensitive histopathological subtypes, it should be mentioned that in breast tumours that express the tyrosine kinase receptor HER2, cannabinoids have been shown to be very effective. In fact, in addition to the correlation between tumour aggressiveness and CB₂ receptor expression in breast cancer, a significant correlation between CB₂ receptor and ErbB2 has been recently demonstrated (Caffarel et al., 2010). In particular, this study showed that the selective agonist JWH-133 was as effective as Δ^9 -THC (a CB₁/CB₂-mixed agonist) in reducing tumour growth and progression through inhibition of the pro-tumorigenic kinase Akt pathway. Moreover, *in vivo* studies reported that Δ^9 -THC was efficacious in reducing tumour growth, tumour number, and the amount/severity of lung metastases in MMTV-neu mice (Caffarel et al., 2010).

Another group of clinically important breast tumours are triple-negative tumours. These tumours are characterized by the total lack of expression in ER, PR or HER2. Numerous pieces of evidence both *in vitro* and *in vivo*, demonstrate that cannabinoids (acting through a plethora of different mechanisms) can be considered as promising candidates for the treatment of ER⁻/PR⁻/HER2⁻ breast cancer. The metabolically stable anandamide analogue (Met-F-AEA), significantly affected adhesion and migration of both the **highly invasive human breast carcinoma cell line**

(MDA-MB-231) and murine breast cancer cell line (TSA-E1) by reducing FAK tyrosine phosphorylation/activation and Src phosphorylation via CB₁ receptor (Grimaldi et al., 2006). Interestingly, the same group reported that pre-treatment with SR141716 could also inhibit tumour growth or induce apoptosis possibly through an inhibition of ERK1/2 signalling in lipid rafts and caveolae (Sarnataro et al., 2006). Both are highly implicated in tumour growth and metastasis in breast cancer (Sloan et al., 2004; Williams et al., 2004). Later studies reported an over-expression of cannabinoid receptors in primary human breast tumours compared with normal breast tissues, as well as in breast cancer cell lines MDA-MB231 and MDA-MB468 (Qamri et al., 2009). Such studies have shown that stimulation of both CB₁ and CB₂ receptor by their agonists, WIN-55,212-2 and JWH-133, respectively inhibited cell proliferation and migration in breast cell lines. The results were in line with *in vivo* findings where in mammary gland tumours in the polyoma middle T oncoprotein (PyMT) transgenic mice model, a genetically engineered model of triple negative breast cancer, WIN-55,212-2 or JWH-133 showed a reduction in tumour growth and lung metastasis. Inhibition induced by the agonists was sensitive to antagonism by CB₁ and CB₂ antagonists AM 251 and SR144528, suggesting involvement of CB₁ and CB₂ receptors.

In terms of signal transduction pathways, cyclooxygenase-2 and prostaglandin E₂, via the regulation of GTPases and transcription factors, have been implicated in the action of cannabinoids on breast cancer growth and metastasis (Qamri et al., 2009). Such studies also reported a significant reduction in angiogenesis (CD31 staining) and attenuation of proliferation (Ki67 staining). A reduction in the level of Cdc42 activity and nuclear expression of transcription factors c-Jun and c-Fos in MDA-MB231 cells were also reported (Qamri et al., 2009).

The involvement of CB₁ as well as TRPV1 receptors on the invasiveness of MDA-MB-231 cells has been recently discussed. Selective agonists were shown to reduce cell invasion and accordingly MMP-2 expression (Farsandaj et al., 2012). In addition, a down-regulation of vascular endothelial growth factor and concomitantly over-expression of COX-2 were also reported

(Farsandaj et al., 2012). In addition, phytocannabinoids were shown to be as effective as synthetic compounds (Ligresti et al., 2006). Particularly, CBD was reported to be effective at inhibiting cell proliferation of both hormone-sensitive (MCF-7) and hormone-negative (MDA-MB-231) cells showing a combination of cell type dependent mechanisms of action which include either direct or indirect activation of CB₂ and TRPV1 receptors and induction of oxidative stress, all contributing to induce apoptosis. The efficacy of CBD was also corroborated with *in vivo* data in athymic mice injected with human MDA-MB-231 breast carcinoma cells (Ligresti et al., 2006). Indeed, it was also shown that in MDA-MB231, a human breast cell line, CBD induced endoplasmic reticulum stress, inhibition of AKT/mTOR pathway, and up-regulation of autophagy-mediated cell death (Shrivastava et al., 2011). **Recent studies indicated** an anticancer activity induced by a quinone/cannabinoid derivative through the activation of CB₂ receptors and oxidative stress mechanism to induce apoptosis in triple-negative breast cancer, a highly aggressive type of breast cancer (Morales et al., 2015). **Other recent studies indicated** that CBD significantly inhibits epidermal growth factor (EGF)-induced proliferation and chemotaxis of breast cancer cells. In addition, it was shown that CBD inhibits EGF-induced activation of EGFR, ERK, AKT and NF-κB signaling pathways as well as MMP2 and MMP9 secretion (Elbaz et al., 2015). CBD also inhibited tumour growth and metastasis in different mouse model systems. Analysis of molecular mechanisms revealed that CBD significantly inhibits the recruitment of tumour-associated macrophages in primary tumour stroma and secondary lung metastases (Elbaz et al., 2015). Figure 3 summarizes the important pathways in cannabinoid induced cytotoxicity.

4. Cannabinoids and brain cancer

Both CB₁ and CB₂ receptors have been identified in the CNS (Ameri, 1999; Benito et al., 2007; Herkenham et al., 1991; Nunez et al., 2004; Skaper et al., 1996). High density of CB₁ receptors has been reported in different areas of the brain such as in the cortex, cerebellum and hippocampus (Herkenham et al., 1991; Hoffman et al., 2010; Sullivan, 2000; Tsou et al., 1998). The

CB₁ receptor protein is mainly localized in astroglial cells and neurones whereas CB₂ receptors are located on microglial cells (Held-Feindt et al., 2006; Stella, 2004) with possible neuroprotective activity (Cabral et al., 2008; Kreitzer et al., 2009) and in some benign paediatric astrocyte tumours (Ellert-Miklaszewska et al., 2007). The anti-tumour effect of cannabinoids on gliomas, glioblastoma **multiforme** or astrocytoma that are the most frequent class of malignant primary brain tumours, will be discussed below. Although the downstream events by which cannabinoids exert their action are not completely elucidated, it can be generally assumed that they act at least through two mechanisms: induction of apoptosis of tumour cells and/or inhibition of tumour angiogenesis and migration.

The anti-tumour action of two cannabinoid receptor agonists, Δ^9 -THC and WIN-55,212-2, was shown to be mediated by an increase in the level of ceramide leading to the activation of extracellular signal-regulated kinase (ERK1/2) in C6 glioma cells (Galve-Roperh et al., 2000; Guzman, 2003). In addition, positive actions of CP 55-940 include a selective induction of cell death in a hybrid cell line of neuroblastoma plus glioma where consistent anti-proliferative effects were observed (Tomiya and Funada, 2011). It was of interest to note that CP 55-940 had a selective and differential action on C6 compared to the U373 astrocytoma cell lines. Results showed that C6 cells were dying faster than the U373 cells mainly via necrotic mechanisms after being exposed to CP 55-940, whilst U373 cells underwent early apoptosis and displayed a more defined laddering pattern (Ortega et al., 2015). This illustrates that the effects of cannabinoids on cancer cell lines *in vitro* is context specific.

Other studies (Ellert-Miklaszewska *et al.*, 2005) also showed a down regulation of phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), and ERK signalling pathways, and activated pro-apoptotic function of Bad protein, which lead to induction of apoptosis. In similar experiments using the CB₂ receptor agonist JWH-133, apoptosis was induced in glioma cells via enhanced ceramide synthesis *de novo* (Sanchez et al., 2001a; Sarfaraz et al., 2008). However, in addition to ceramide pathway, other pathways such as stress-regulated protein p8 which leads to the

activation of activating transcription factor-4 (ATF-4) and cell death-inducible kinase (TRB3) were shown as mechanism/s of the anti-tumour action of cannabinoids (Carracedo et al., 2006a). Other studies have demonstrated that CBD, via receptor-independent manner, triggers apoptosis of human glioma cells by a cellular mechanism that involves an early production of reactive oxygen species (ROS), depletion of glutathione (GSH), and concomitant activation of caspase cascade with no effect in non-transformed cells (Massi et al., 2006). Moreover, the same group, reported that CBD can inhibit proliferation and invasion of different glioma cell lines through a multi-target mechanism affecting the most relevant pro-tumour ERK and PI3K/Akt signalling pathways, as well as the expression of the transcription factor HIF-1 α which was down following a treatment with CBD. Such experiments were conducted under 'pseudo-hypoxic conditions' (Solinas et al., 2013). This raises the possibility that these compounds could have activity against hypoxic cells but to the best of our knowledge, no studies of this nature have been conducted.

Remarkably, the cannabinoid-mediated anti-proliferative action appears to be selective for brain-tumour cells as the survival of normal brain cells astrocytes (Gomez Del Pulgar et al., 2002), oligodendrocytes (Molina-Holgado et al., 2002) and neurons (Mechoulam et al., 2002) are unaffected or even favoured by cannabinoid challenge. Accordingly, a pilot phase I clinical trial, in a cohort of recurrent glioblastoma multiforme tumour patients expressing cannabinoid receptors reported an anti-proliferative action for Δ^9 -THC on tumour cells with an acceptable safety profile (Guzman et al., 2006). Further phase 1b/2a clinical trials are underway and await publication.

Based on these findings, other studies evaluated the expression of cannabinoid receptors in surgical material of solid astrocytomas, gliomas and glioma cell lines. Whilst CB₁ expression was slightly increased in astrocytomas and gliomas, the CB₂ expression was similar in both tumour and normal brain tissue. The authors found, in accordance with this receptor subtype expression *in situ*, that agonists selective for CB₁ or active on both subtypes reduced elevated cyclic AMP levels and cell proliferation, but failed to induce apoptosis in glioma cells *in vitro* (Held-Feindt et al., 2006).

Further experiments described opposite changes in CB₁ and CB₂ receptor protein expression in human gliomas (Lopez de Jesus et al., 2010). The reduction in the level of CB₁ receptor expression or mRNA in glial tumours is suggested to be related to the neuronal loss (Canoll & Goldman, 2008). The reduction in CB₁ receptor expression was in line with a reduction in the WIN 55,212-2 stimulated [³⁵S]GTPγS binding to glioblastoma multiforme membranes. This suggested a reduction in the number of available receptors in the glioblastomas. Similar observations were reported in the brain of aged rats (Romero et al., 1998). It should be noted that similar reductions were also observed in neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases, and also in normal aging (Glass et al., 1993; Hurley et al., 2003; Richfield et al., 1994; Westlake et al., 1994). In particular the up-regulation of CB₂ receptors has also been reported in other disorders such as Alzheimer's, Huntington's diseases, encephalitis and multiple sclerosis (Benito et al., 2007; Benito et al., 2003; Fernandez-Ruiz et al., 2007). It is suggested that reduction in the level of CB₁ receptors occurs as a result of neuronal loss, possibly simultaneous to the enhanced brain gliosis that appears with normal aging. In addition to an up-regulation of CB₂ receptors in malignant tumours, the level of anandamide was reported to increase in glioblastomas (Petersen et al., 2005). It can then be suggested that the endogenous anandamide will bind to CB₂ receptors to mediate an anti-tumour activity. This is in line with studies by Sanchez et al in 2001 who reported a considerable regression of malignant glioma cells by the local administration of a CB₂ selective agonist (Sanchez et al., 2001a). In such studies administration of JWH-133 to an immunocompromised mice model, Rag-2^{-/-} mice inoculated with rat glioma C6-cells caused a 71% reduction in the tumour growth as compared to the control group and was found to be prevented by co-administration of the CB₂ receptor antagonist, SR144528 but not the CB₁ receptor antagonist, SR141716. Further experiments by the same group indicated that the anti-tumour effects induced by the activation of CB₂ receptors by JWH-133 initiated apoptosis via ceramide synthesis and ERK1/2 activation (Sanchez et al., 2001a). In later studies by Blazquez and colleagues in 2003 using the same mouse model, intra-tumour administration of JWH-133 showed a significant reduction in

mRNA expression of the pro-angiogenic factors, vascular endothelial growth factor and angiopoietin 2 (Blazquez et al., 2003). Additional data to support the importance of CB₂ receptors in glioblastoma came from studies that showed an increase in the level of CB₂ receptors in the endothelial cells of human glioblastoma vessels (Schley et al., 2009). Whilst the mechanism of action of CB agonists against glioblastoma is still not fully understood, it has been shown in the rat C6 glioma cells that the high affinity glycine transporter (GLYT1) has been attenuated via protein kinase C alpha (PKC- α) (Morioka et al., 2008). Attenuation of GLYT1 would increase the inhibitory action of glycine transmitter in the synapse. Other studies showed that PKC inhibitors could impair the CB effects in neuroblastoma cells (Rubovitch et al., 2004). The above data suggest the involvement of downstream PKC and GLYT1 regulation in mediating an anti-glioblastoma activity by cannabinoids. Thus, the accumulated data suggest that the anti-tumour activity of cannabinoids could be mediated via ERK1/2, AKt and/or PI3K pathways as well as PKC and glycine transporters. The change in the levels of cannabinoid receptor expression and the levels of endocannabinoids depend on the stages of cancer. For example, the levels of CB₂ receptors and anandamide increase in advanced tumours.

5. Cannabinoid and lung cancer

The first evidence of the antineoplastic activity of cannabinoids against lung cancer dates back to 1975 when Munson et al demonstrated a dose-dependent retardation in tumour growth in the Lewis lung adenocarcinoma animal model (Munson et al., 1975). Later on, further studies were carried out in order to elucidate the possible mechanism of action(s) of this class of molecule although controversial evidence about the anti-tumour action of cannabinoids were reported for this particular type of cancer. In fact, it has been reported that Δ^9 -THC suppresses the host immune reactivity against lung cancer and that the augmentation of tumour growth acts through inhibition of anti-tumour immunity by a CB₂ receptor-mediated, cytokine-dependent pathway (Zhu et al., 2000). Δ^9 -THC, although without the modulation of EGFR expression or FAK phosphorylation previously

reported by others (Hart et al., 2004), has been also shown to attenuate the EGF-induced migration and invasion of epidermal growth factor receptor-overexpressing lung cancers, which are often highly aggressive and resistant to chemotherapy (Preet et al., 2008). Furthermore, in *in vivo* experiments, administration of Δ^9 -THC suppressed metastasis and subcutaneous tumour growth in severe combined immunodeficient mice (Preet et al., 2008). The anti-invasive effect of cannabinoids has also been reported (Ramer et al., 2008 and 2010). In these studies, a cannabinoid receptor and TRPV1-triggered expression of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) was identified as an important mediator of the anti-invasive action of cannabidiol. Impaired invasion driven by CBD links the cannabinoid and TRPV1 receptors to the activation of MAPK pathways and subsequent TIMP-1 induction. Additionally and in line with its *in vitro* anti-invasive action, *in vivo* studies in thymic-aplastic nude mice revealed a significant inhibition of A549 lung metastasis in cannabidiol-treated animals as compared to vehicle-treated controls (Ramer et al., 2008 and 2010).

The expression of CB₁ and CB₂ receptors has been reported in non-small cell lung cancer (NSCLC) patients and also the NSCLC cell lines, A549 and SW-1573 (Preet et al., 2011). Pre-treatment of A549 and SW-1573 cells with WIN 55,212-2 and JWH-015 reduced chemotaxis and chemo-invasion as well as migration and these were sensitive to blockade by the CB₁ and CB₂ receptor antagonists, AM251 and AM630 respectively. Furthermore both agonists revealed a reduction in tumour growth *in vitro*, inhibited *in vivo* tumour growth and lung metastasis that again were sensitive to antagonism of CB₁ and CB₂ receptors (Preet et al., 2011). Mechanistic studies revealed an inhibition of phosphorylation of Akt and MMP-9 expression/activity upon exposure to the CB₁ and CB₂ receptor agonists in NSCLC (Preet et al., 2011). On the other hand, the involvement of cannabinoid receptor is not the only mechanism through which cannabinoids exhibit their anti-tumor properties. In fact, as demonstrated by Gardner et al, these molecules can exert some of their biological effects via modulation of prostaglandin production. This study has shown that administration of methanandamide at 5.0 mg kg⁻¹ in murine lung cancer could increase the rate

of tumour growth (both *in vitro* and *in vivo*). Such studies also showed an increase in the level of prostaglandin (PG) E₂ and COX-2 which were sensitive to blockade by the COX-2-specific inhibitor, SC58236, the p38/MAPK inhibitor, SB203528, and the p42/44 inhibitor, PD98059 but not to CB₁ and CB₂ receptor antagonists. The results confirmed an up-regulation of COX-2 which was independent of cannabinoid receptor activation (Gardner et al., 2003). Recently studies have shown an up-regulation of COX-2 induced by CBD in two different lung cancer cell lines (A549, H460) and primary cells from a patient with lung cancer (Ramer et al., 2013). Such studies showed a pro-apoptotic and tumour-regressive action by CBD through an initial up-regulation of COX-2 and PPAR- γ and a subsequent nuclear translocation of PPAR- γ by COX-2-dependent prostaglandins, PGD₂ (Ramer et al., 2013). Such studies also indicated that incubation of the cells with PGD₂ but not PGE₂ was associated with a concentration-**dependent** loss of cell viability. This highlighted how different cannabinoids could induce differential effects, which are CB₁ and CB₂ receptor –independent pathways and also **dependent** on the type of PG being produced.

In further recent studies, it was demonstrated that cannabinoids induced up-regulation of intercellular adhesion molecule 1 (ICAM-1) on lung cancer cells to be responsible for increased cancer cell lysis by lymphokine-activated killer (LAK) cells (Haustein et al., 2014). This recent study suggested a new mechanism for anti-tumour activity of cannabinoids. Further experiments by Ramer et al also indicated that cannabinoids induce anti-angiogenic effect in endothelial cells via the release of TIMP-1 from lung cancer cells (Ramer et al., 2014).

Thus, the likely pathways involved in mediating anti-tumour activity induced by cannabinoids are CB₁, CB₂ and TRPV1 receptors as well as MAPK and TIMP-1 pathways. However, this does not negate a role for PPAR- γ and COX-2-dependent prostaglandins.

6. Cannabinoids and intestinal cancer

Endocannabinoid signalling has been proved crucial for certain aspects of gastrointestinal homeostasis. It also plays an essential role in the regulation of intestinal tumour growth. Recent

studies have demonstrated an up-regulation of anandamide and its metabolite arachidonic acid in cancer tissues of patients with colon cancer with lymphatic metastasis (Chen et al., 2015). In addition, CB₁ receptor expression was elevated (Chen et al., 2015). Another recent study also demonstrated that the level of expression of CB₂ receptors correlates with cancer progression and can predict patient survival in colon cancer patients. Such studies showed that high levels of CB₂ receptors correlate with poor prognosis in patients with tumours in advanced stages or with vascular invasion (Martinez-Martinez et al., 2015).

There are however several controversies regarding the role of the cannabinoid receptors in colorectal cancer (Izzo et al., 2009). In adenomatous polyposis coli (APC) gene knock-out models (mutation of the gene leads to colon cancer), mice with an additional deletion in the cannabinoid receptor 1 (CNR1) gene or subjected to pharmacological blockade of the CB₁ receptor, demonstrated a higher colonic tumour burden than their littermates whereas activation of CB₁ attenuated intestinal tumor growth by inducing cell death via down-regulation of the anti-apoptotic factor survivin (Wang et al., 2008). In contrast to these findings, the CB₁ antagonist rimonabant inhibited the growth of cancer cells and the development of precancerous lesions in mice (Santoro et al., 2009). **Other studies** showed that non-selective cannabinoid receptor agonists such as anandamide, 2-AG and HU-210, and an inhibitor of anandamide inactivation, potently inhibited human epithelial colorectal adenocarcinoma cells (CaCo-2 cell) proliferation (Ligresti et al., 2003). This effect was less prominent in a less aggressive human colon carcinoma cell line (DLD-1 cells). The cell proliferation effect afforded by HU-210 was inhibited by the CB₁ and CB₂ receptor antagonists, rimonabant and SR144528 respectively, only in DLD-1 cells and not in CaCo-2 cells. This suggested the involvement of both CB₁ and CB₂ receptors in mediating an inhibition of cell growth (Ligresti et al., 2003). The differential effects observed in the two different cell lines might be due to differences in the level of cannabinoid receptor expression. Indeed, it was noted that CaCo-2 cells express CB₁ receptors but not CB₂ receptors and DLD-1 cells express both CB₁ and CB₂ receptors, with CB₁ receptor less expressed than in CaCo-2 cells. Later studies suggested that

cells with high expression of cyclooxygenase-2 (COX-2) might be a target for the inhibitory action of anandamide on cell death in colorectal carcinoma cells (Patsos et al., 2005). Indeed CB₂ receptor expression has been reported in human adenomatous polyps and carcinomas and in human colonic epithelial cell lines (Greenhough et al., 2007; Ihenetu et al., 2003; Ligresti et al., 2003; Wright et al., 2005). Interestingly the normal epithelial cells do not express CB₂ receptors (Wright et al., 2005). This suggests that the CB₂ receptors are inducible in inflamed tissues or tumour cells. This suggestion is in line with other studies which reported an increase in the level of CB₂ receptor expression associated with increased differentiation, proliferation, disease and malignancy (Fernandez-Ruiz et al., 2007; Mallat et al., 2008). Ceramide synthesis following an increase in the level of tumour necrosis factor (TNF)- α and activation of epidermal growth factor receptor have been identified as the possible molecular mechanism following the activation of CB₂ receptors in colon cancer (Cianchi et al., 2008; Hart et al., 2004). Thus since activation of CB₂ receptors seem to be beneficial in cancer therapy, further studies are needed to investigate the dual role for CB₂ receptors in intestinal regeneration and anti-tumour activity. It is also possible that different subtypes of CB₂ receptors mediate different roles, however no evidence has yet been reported.

Cannabinoids have been also reported to exert chemopreventive effects in an experimental model of colon cancer, an effect associated with down-regulation of phospho-Akt and up-regulation of caspase-3 (Aviello et al., 2012). *In vitro* studies by the same group on colorectal carcinoma cells demonstrated that anti-proliferative effects were exerted through multiple mechanisms, including involvement of CB₁ receptors, TRPV1 and PPAR- γ (Aviello et al., 2012). Moreover, studies by Notarnicola and collaborators firstly described the up-regulation of CB₁ expression by 17 β -estradiol as a further mechanism by which estrogens control colon cancer cell proliferation (Notarnicola et al., 2008). Proto and colleagues confirmed these findings and reported an interaction between the endocannabinoid system and steroid hormones in the growth of colon cancer cells. Such studies revealed that both anandamide and 17 β -estradiol inhibited proliferation of human colorectal cancer cell lines, SW620 and DLD-1 via interaction with CB₁ receptors. Both agonists increased the CB₁

receptor expression in both cell lines by acting at the same CNR1 gene. Interestingly the up-regulation of CB₁ receptors induced by anandamide analogue was through PPAR- γ and RXR α , (Proto et al., 2012). The data suggested that CB₁ receptor is a target for 17 β -estradiol and that the endocannabinoid system could present a tool to improve treatment in patients with colorectal cancer.

7. Cannabinoids and reproductive system cancer

During the last decade, increasing evidence has pointed towards the relevance of endocannabinoids in both female and male fertility. This association has been supported by the tightly modulated expression of cannabinoid receptor found in gonadal tissues. Along the male reproductive tract, CB receptors have been detected in the testis, Sertoli cells, prostate and vas deferens (Gye et al., 2005; Maccarrone et al., 2003; Pertwee et al., 2002; Rossato et al., 2005). CB receptors have also been found in various parts of the mammalian female reproductive system. In the mouse reproductive tract, CB receptors were expressed in the uterus, oviduct and also in pre-implantation embryos (Das et al., 1995; Paria et al., 2001; Wang et al., 2004). Moreover, this localization has also been described in the human uterus (Dennedy et al., 2004; Iuvone et al., 2008) and placenta during pregnancy (Habayeb et al., 2008; Helliwell et al., 2004; Park et al., 2003).

The influence of cannabinoids on the proliferation of human cervical adenocarcinoma cells and on macromolecular biosynthetic events associated with the proliferative process were reported in the late 1970s when cannabinoids induced growth inhibition of HeLa cells, human cervical cancer cell lines (Blevins et al., 1980; Mon et al., 1978). More recently, an up-regulation of both cannabinoid receptors in human ovarian cancer cells OVCAR-3 and SKOV-3 compared to normal Chinese hamster ovarian (CHO) cells was found. These findings led to the suggestion that these are targets for new therapies for ovarian cancer (Afaq et al., 2006). Recently studies also showed that CB₁ receptor levels are also increased and correlate with disease severity in human epithelial ovarian tumours and this has been proposed to be an important factor of bad prognosis following surgery in

stage IV colorectal cancer (Messalli et al., 2014; Jung et al., 2013). WIN-55,212-2 was shown to exert, via CBR dependent manner, a decrease in cell viability, G1 arrest in cell cycle progression, induction of apoptosis and down-regulation of the expression of PCNA and VEGF (Afaq et al., 2006). An abnormal expression of CB₂ receptor has also been reported in biopsies of women affected by endometrial carcinoma. Interestingly, the up-regulation was only found in transformed malignant cells and the staining of CB₂ was completely absent in the normal endometrial tissue from the same biopsy (Guida et al., 2010). These findings, together with previous evidence that the endocannabinoid system controls cell survival/death decisions (Guzman et al., 2002) by inhibiting or stimulating cell growth, suggest that CB₂ receptors might play an important role in the growth of endometrial carcinoma. The study revealed that the complete endogenous machinery for CB₂ activation was altered in endometrial adenocarcinoma, because the levels of 2-AG, the most efficacious endogenous CB₂ agonist, were elevated, possibly as a result of the decrease in the expression of monoglycerol lipase (MAGL) an important enzyme necessary for 2-AG breakdown. On the other hand, CB₁ receptors and AEA, a more selective endogenous agonist for CB₁, as well as FAAH, the most important AEA-metabolizing enzyme, although expressed in healthy endometrial tissues, remained unchanged after cell transformation (Guida et al., 2010).

Additional proteins other than cannabinoid receptors have been considered as possible targets in reproductive cancers. Multidrug resistance-related protein 1 (MRP1) or ATP-binding cassette (ABC) transporter, ABCC1, is a membrane-bound, ubiquitously expressed energy-dependent efflux transporter. In terms of physiological function, it is involved in transporting a range of glutathione, glucuronide, sulfate conjugates and cancer drugs, including folate based anti-metabolites, anthracyclines, plant-derived vinca alkaloids and anti-androgens (Cole et al., 1994; Flens et al., 1996; Hooijberg et al., 1999; Keppler et al., 1997). Whilst the transportation of metabolites by ABCC1 leads to attenuation of the toxicity of such metabolites might be beneficial, efflux of cancer drugs would however reduce intracellular concentrations in tumour cells and hence induce drug resistance (Karászi et al., 2001; Norris et al., 1996; Wijnholds et al., 2000; Wijnholds et

al., 1998). It has been shown that phytocannabinoids are modulators of the ABC transporters, ABCG2 and P-glycoprotein (Holland et al., 2007; Zhu et al., 2000). It was also shown in the human ovarian carcinoma cell line that cannabinoids such as cannabitol, cannabidiol and Δ^9 -THC increased the intracellular accumulation of two ABCC1 substrates, Fluo3 and the cancer drug, vincristine, in 2008/*MRP1* cells (the human *ABCC1* transduced subline) (Holland et al., 2008). In such experiments cannabidiol was shown to be the most potent and Δ^9 -THC to be the least potent cannabinoid. The rank order of potency for ABCC1 inhibition was independent of the substrate assayed (Holland et al., 2008). Further pre-clinical studies are required to establish if inhibition of ABC transporters by cannabinoids can alter the disposition and efficacy of therapeutic drugs that are substrates for these transporters.

With regard to the male reproductive system and its physiology, the antagonizing effect of cannabinoids can be dated back to 1974 where experimental models in male rats showed depression of spermatogenesis (Dixit et al., 1974) and decrease in circulating testosterone levels (Kolodny et al., 1974). Endocannabinoids, through interaction with CB₁ receptors and synthetic endocannabinoid-vanilloid hybrids via stimulation of TRPV1 channels have been shown to inhibit nerve growth factor (NGF)-induced proliferation of human prostate PC-3 cells (Melck et al., 2000). However, THC was suggested to induce apoptosis of these cells via a receptor-independent mechanism (Ruiz et al., 1999), but also increase the production of the pro-proliferative factor, NGF (Velasco et al., 2001). Later studies showed an increased expression of both CB₁ and CB₂ receptors in cultured prostate cancer cells when compared with normal prostate cells. Moreover, treatment of prostate cancer cells with WIN-55,212-2 resulted in a dose and time dependent decrease in cell viability, increased apoptosis along with decrease in androgen receptor protein expression, PSA expression, and secreted PSA, suggesting that cannabinoids should be considered as agents for the management of prostate cancer (Ruiz-Llorente et al., 2003; Sanchez et al., 2003; Sarfaraz et al., 2005). It was also found that a high level of CB₁ receptor immunoreactivity (CB₁IR) in prostate cancer tissues is associated with the severity and outcome of the disease (Chung et al., 2009).

It was then suggested that the effect of cannabinoids on prostate cancer cells depends on the concentration of cannabinoids used and the incubation time. Cannabinoids used at concentrations lower than micromolar induced androgen receptor expression whilst at higher concentrations induced apoptosis or cell-cycle arrest (Mimeault et al., 2003; Sanchez et al., 2003; Sarfaraz et al., 2006). It was also shown that CB₁ receptor antagonists prevented/blocked the anti-tumour activity of agonists whilst a longer incubation time failed to reveal any antagonist effect (Mimeault et al., 2003; Sarfaraz et al., 2005).

Other studies have also indicated an important role for CB₂ but not CB₁ receptors in the anti-proliferative effect of cannabinoids (Olea-Herrero et al., 2009). Such studies have shown that in PC-3 cells pre-treated with rimonabant failed to reduce the effect of methanandamide on cell cycle and apoptosis. However, pre-treatment with the CB₂ receptor antagonist, SR 144528 attenuated the number of apoptotic cells and the number of sub-G1 cells induced by methanandamide and apoptosis afforded by the CB₂ receptor agonist, JWH-015. Furthermore, when CB₂ receptor expression was significantly reduced by siRNA, apoptosis afforded by JWH-015 was completely antagonised thus further indicating an involvement of CB₂ receptors in apoptosis (Olea-Herrero et al., 2009).

Recent studies have focused on the role of non-selective, calcium permeable cation channels of the transient receptor potential (TRP) channels in prostate cancer initiation and progression. Capsaicin, a natural ligand for TRPV1, has been reported to elicit both pro-proliferative and pro-apoptotic effects on prostate cancer cell lines (Czifra et al., 2009; Malagarie-Cazenave et al., 2009; Malagarie-Cazenave et al., 2011; Sánchez et al., 2006; Sanchez et al., 2005; Ziglioli et al., 2009). Moreover, it has been suggested that TRP channels of melastatin-type 8 (TRPM8) are over-expressed in androgen-dependent prostate cancer cell lines in a manner dependent on androgen receptor (AR) activation (Bidaux et al., 2007; Bidaux et al., 2005; Henshall et al., 2003; Tsvaler et al., 2001; Zhang et al., 2004). Several studies have shown that cannabinoids antagonize TRPM8 channels and activate and subsequently desensitize TRPV2 and TRPV1 channels (De Petrocellis et

al., 2011; De Petrocellis et al., 2008; Qin et al., 2008). Further recent studies showed an inhibition of cell viability and induction of apoptosis by cannabinoids when tested in serum protein-deprived medium suggesting an intracellular target for the cannabionids (De Petrocellis et al., 2013). However, the molecular mechanism of action was demonstrated to be due not uniquely to a direct TRPM8 antagonism, but rather to AR down regulation, which in turn can lead to TRPM8 down-regulation. As it has been suggested that oestrogens are involved in the survival of prostate cells, the authors also examined the involvement of those receptors and they found that GPR30, rather than oestrogen metabolic enzymes or ER α or ER β , may be one of the intracellular targets through which cannabinoids stimulate the ER branch of the intrinsic pro-apoptotic pathway Δ^9 -THC (De Petrocellis et al., 2013).

In addition, the expression of a potential cannabinoid receptor, GPR55 has been also shown at the mRNA and protein level in both ovarian and prostate cancer cell lines (Pineiro et al., 2011). Although the physiological role of this receptor is not fully understood, it is suggested to have important roles in regulating proliferation and growth (Pineiro et al., 2011). This novel receptor is suggested to be associated with lysophosphatidylinositol (LPI) to induce calcium mobilization and activation of Akt and extracellular signal-regulated kinase (ERK)1/2 in ovarian and prostate cell lines. Blockade of this receptor might have novel therapeutic potential in managing ovarian and prostate cancer. All these findings indicate that cannabinoids can retard proliferation and cause apoptosis of PCC via a combination of cellular and molecular mechanisms (cannabinoid receptor-mediated and/or independent). These studies are encouraging and they support the development of clinical studies on these molecules as a therapy for human prostate carcinoma, either as single agent or in combination with existing compounds.

8. Conclusion and Future Direction

The substantial knowledge of palliative and anti-tumour actions of cannabinoids gained by the scientific community in the last few years has raised the profile of these molecules and many are promising candidates for cancer treatment. However, the use of cannabinoids in medicine is limited by their psychoactive effects, thus cannabinoid-based therapies that are devoid of unwanted side effects or with a safe profile/pharmacological window are required. A further aspect which complicates the practise of cannabinoid-based therapies is the lack of detailed understanding of their mechanisms of action. There is plenty of evidence in literature, mostly of them reported in this review, about the ability of cannabinoids to induce different pathways of cell death depending on the neoplastic cell type under investigation. On the other hand, the effect of these compounds on several distinct hallmarks of cancer rather than on one single process is potentially desirable. Even though the resolution of the conflicting evidence around cannabinoid action still remains a high research priority, some key points need to be emphasized. Despite a small number of reports that state their inefficacy, the vast majority of independent pre-clinical studies report a sustained anti-tumour activity for cannabinoids. Moreover, an important feature of cannabinoid pharmacology that can have important clinical implications is the lack of toxicity frequently reported on non-tumour cells. Cannabinoid-based medicines have been already proven to be safe in thousands of patients enrolled in clinical trials for cancer patients (Grotenhermen, 2007; Portenoy *et al.*, 2012; Robson, 2011). This highlights a need for the identification of the molecular mechanisms which confer sensitivity to this class of drugs. Another appealing possibility consists of adding to a standard therapy a mixture of molecules able to directly or indirectly target the endocannabinoids system in order to enhance anti-tumour actions of chemotherapy and attenuate unwanted iatrogenic side effects. Indeed it is interesting to note that the administration of THC and CBD enhanced the radio-therapeutic effect in an orthotopic murine glioma model (Scott *et al.*, 2014). It is proposed that CBD could alleviate the THC-induced side effects such as convulsion, dis-coordination, and psychotic episodes, thus it was suggested that the administration of CBD in combination with THC may help to improve the tolerability to cannabinoids (Pertwee, 2009). Finally, only the improvement of basic

research will lead to the identification of the most appropriate patient population for a cannabinoid-based therapy and will facilitate the acceptance of cannabinoid use in the clinic.

Conflict of interests/acknowledgement

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Figure 1. Chemical structures of some cannabinoids.

Table 1. Tumours, which are sensitive to cannabinoid-induced growth inhibition.

Figure 2- Schematic representation showing some examples of pathways activated following the activation of cannabinoid receptors.

Figure 3. Schematic representation of examples of different pathways associated with anti-proliferative effects induced by cannabinoid receptor activation in breast cancer.