

Review Article

Cannabinoids as an Anticancer Agent for Prostate Cancer

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OPEN ACCESS**Abstract**

Over the past decade, the endocannabinoid system has emerged as a novel target for the treatment and prevention of cancer and various diseases. Cannabinoids consist of the active components of the plant *Cannabis sativa*, and can be classified into three groups; phytocannabinoids, endocannabinoids, and synthetic cannabinoids. Although mainly used as an antiemetic and for cancer-related pain, recent findings have revealed antiproliferative and anti-metastatic effects in various cancer models. Overexpression of cannabinoid receptors in malignant prostate tissue suggests an association between the endocannabinoid system and prostate regulation, thus proposing potential therapeutic opportunities for prostate cancer. We conducted a systematic review that highlights the potential anticancer effect of cannabinoids in prostate cancer using both *in vitro* and *in vivo* models. A hand-search was run on PubMed database from 1997 to January 2017 for relevant studies. Results detail potential apoptotic and anti-metastatic effects through pathways involving endoplasmic reticulum stress, oxidative stress and Rho GTPase signalling. These observations have contributed to our understanding of the role of cannabinoids in cancer progression; however further analysis on the pharmacodynamics is warranted, including molecular cross links between cannabinoids and available chemotherapeutic drugs.

Keywords

- Prostate cancer
- Cannabinoid
- Anticancer
- Apoptosis
- Anti-migration

ABBREVIATIONS

PCa: Prostate Cancer; CB: Cannabinoid receptor; THC: ⁹-Tetrahydrocannabinol; CBD: Cannabidiol; pCB: Phytocannabinoid; FAAH: Fatty Acid Amide Hydrolase; AEA: Anandamide; 2AG: 2-Arachidonoylglycerol; SC: Synthetic Cannabinoid; RCT: Randomized Control Trial; IP: Intraperitoneal; TRAMP: Transgenic Adenocarcinoma Model of the Mouse Prostate; ER: Endoplasmic Reticulum; UPR: Unfolded Protein Response; mTOR: Mammalian Target of Rapamycin; ROS: Reactive Oxygen Species; GPCR: G Protein Coupled Receptor

INTRODUCTION**Overview of prostate cancer**

Prostate cancer (PCa) is the most common malignancy among Canadian men and the third leading cause of cancer related death in men. It is estimated that one in eight Canadian men are expected to develop prostate cancer during their lifetime and one in 27 will die from the disease [1].

Advances in screening and diagnostic techniques have led to more frequent diagnoses of prostate cancer [2]. While localized disease can be effectively treated by various modalities, therapeutic approaches for advanced stages of PCa are limited. Most treatment options depend upon tumor characteristics, patient status and age. Although these options increase

patient longevities, and in some cases, may cure cancer, they unfortunately affect both cancerous and healthy cells, thus are associated with adverse side effects, including urinary incontinence, erectile dysfunction and reduced quality of life [3]. Strategies that minimize the morbidity and mortality is necessary to reduce the burden of this disease.

Cannabinoids

Cannabis has been used for medicinal purposes dating back more than 5000 years. Since the discovery of cannabinoid receptors and their endogenous ligands, the amount of research on the physiology and therapeutic benefit of cannabinoids has grown [4]. Cannabinoids can be classified into three groups based on the source of their production; phytocannabinoids, endogenous cannabinoids, and synthetic cannabinoids. Their effects are mainly mediated via the activation of two G-protein coupled cannabinoid (CB) receptors, CB₁ and CB₂. The endogenous ligands for these receptors are synthesized on demand and exert similar effects to ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD), the main psychoactive components of cannabis, however their effects are short-lived due to effective metabolic pathways [5,6]. The endogenous cannabinoids, their receptors, and the enzymes responsible for their synthesis, transport, and degradation, together make up the endocannabinoid system. This system is crucial for neuromodulatory activity, control of cardiovascular

tone, energy metabolism, immunity, and reproduction, thus making it a promising target for the management of a variety of diseases.

Phytocannabinoids

Phytocannabinoids (pCBs) are lipid-soluble phytochemicals occurring naturally in the plant, *Cannabis sativa* L, and include the main psychoactive constituents, THC and CBD. THC acts as a partial agonist at the CB receptors, and most of its psychoactive effects are mediated by activation of the CB₁G-protein coupled receptor. pCBs are used to treat anorexia in people with HIV and as an antiemetic in people undergoing chemotherapy. Studies have suggested that CBD exerts some of its pharmacological activity through the inhibition of fatty acid amide hydrolase (FAAH), which subsequently increases the levels of endogenous cannabinoids [7]. Several pCBs have been reported to bind to and interact with CB receptors at high affinities, appearing to be promising candidates for drug development and cancer therapeutics [8]. One of such promising treatments is Nabiximols, trade name Sativex; currently available as an oral mucosal spray with a one to one ratio of THC to CBD. Sativex is a pharmaceutical product approved for its use in the treatment of neuropathic pain in multiple sclerosis and cancer induced pain. Phase I clinical trials have recently been launched for glioblastoma multiforme, whereby Sativex is used in combination with temozolomide, standard of care chemotherapy for brain cancer. As the first study to examine cannabinoids in combination with chemotherapy, this research will highlight potential side effects of cannabinoids and their interactions with chemotherapeutic agents, paving the way for their use in a variety of cancers.

Endocannabinoids

Endocannabinoids are compounds produced in our body that bind to CB receptors. They act as neuromodulators, affecting the release of various neurotransmitters in the periphery, and play a vital role in inflammation, and fat and energy metabolism [9]. Two of the best-studied endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2AG). AEA and 2AG are mainly metabolized by FAAH and monoacylglycerol lipase, respectively. While 2AG is prevalent at relatively high levels in the nervous system, AEA is present at very low levels due to high metabolic breakdown rates, and is produced on demand rather than stored in intracellular compartments. The role of endocannabinoids in cancer has been implicated in studies depicting inhibition of cancer cell proliferation *in vitro* and *in vivo* [10]. In colon cancer, endocannabinoid treatment has shown inhibition of colonic inflammation, and this effect was reversed by deletion of CB receptors [11-13]. The dysregulation in cannabinoid receptors suggests their involvement in the malignant transformation of the colon from a healthy to a diseased state. Despite limited clinical use due to rapid metabolism, endocannabinoids will remain useful for uncovering the dynamics between the endocannabinoid system and a variety of disease states.

Synthetic cannabinoids

Synthetic cannabinoids (SC) have been extensively used as research tools to gain insight into the endogenous cannabinoid system and to assess therapeutic use. A majority of SCs have a greater binding affinity to the CB receptors compared to pCBs. *In vitro* and *in vivo* studies have shown that SCs analgesic, anti-inflammatory, and anticancer growth effects are approximately

two to 100 times more potent than THC [14]. However, depending on target tissue, route of administration, doses, and duration of treatment, they show both anticancer and protumoral activity [15]. Several studies focus on CP55940, a non-classical cannabinoid, WIN55212, an aminoalkylindole, as well as JWH cannabinoids. CP55940 is an SC that mimics the effects of THC and is currently being used to study the endocannabinoid system. WIN55212 also mimics the effects of THC; however, it has a different chemical structure and a much higher affinity for the CB₂ receptor compared to THC. JWH cannabinoids such as JWH-007, JWH-015, JWH-018, and JWH-030 are from the naphthoylindole family and act as selective CB receptor agonists [16].

Since the legal banning of *Cannabis sativa* in Canada in 1923, cannabinoids have become quite controversial; frowned upon for their recreational uses. However, with recent medical research focusing on the endocannabinoid system as a potential therapeutic target, cannabinoids are becoming recognized as key mediators of several aspects of human health and disease [17]. Evidence from a multicenter randomized control trial (RCT) has shown that cancer patients treated with oral cannabis showed analgesic efficacy in the low and medium dose ranges [18]. Additionally, spasticity, spasm frequency, insomnia, pain, and impaired mobility in patients with multiple sclerosis, showed significant improvement in a multicenter RCT involving 630 subjects over a 12-month period [19]. Although cannabinoids can be effective for symptom management in palliative care, prescribing them to patients becomes an issue as exact dosages depend on patient needs and tolerance to drug use. Despite this, evidence from numerous studies has suggested that therapeutic agents targeting the cannabinoid receptors may be promising for the treatment of a variety of cancers. This review will outline current evidence relating to the anti-proliferative and anti-migratory potential of cannabinoids and will discuss signalling pathways modulated by cannabinoids in prostate cancer cells.

METHODS

Search criteria

PubMed database was searched for relevant studies published from 1997 to January 2017. Key words used in the search included "cannabinoids", "cannabis", or "endocannabinoids" AND "prostate cancer". Studies were selected based on initial screening of title and abstract and potentially relevant articles were identified for full-text review.

Selection of studies

A study was eligible for inclusion if it outlined an association between cannabinoid receptor activation and effect on prostate cancer. All study types were included, except for case studies, commentaries, and expert opinions.

Data abstraction

Data abstraction was performed with focus on identifying anti-proliferative, anti-migratory, or apoptotic effects of cannabinoids *in vitro* in prostate cancer, effect of cannabinoid administration on tumor size *in vivo*, and the molecular targets mediated by cannabinoids.

RESULTS

Our search strategy resulted in an initial identification of 79 studies. After title and abstract screening, and review for

eligibility, 21 studies were included for final analysis.

Role of cannabinoids in prostate cancer

Recently, the therapeutic potential of cannabinoids in oncology has expanded from its palliative actions and has moved towards its antitumor effects in a wide range of cancers [20]. Studies have proposed that cannabinoids contribute to maintaining a balance in cell proliferation. It is suggested that targeting the endocannabinoid system will thereby affect the growth of a variety of cancers, including breast, brain, skin, thyroid, prostate and colorectal cancers. Additionally, it has been reported that cannabinoid receptors are overexpressed in prostate, breast, skin, and hepatocellular carcinoma [21,22]. Focusing specifically on prostate cancer, Chung et al. [3], demonstrated that CB₁ receptors and FAAH are over expressed in tumor tissue compared to non-malignant epithelium. Additionally, these investigators showed an association between high CB₁ expression, disease severity and prognosis. In this context, they observed a correlation between CB₁ expression and disease outcome in tumor tissue. However, this was not observed in non-malignant tissue, suggesting it to be a local change rather than a generalized underlying issue. Over expression of components of the endocannabinoid system in tumors of increasing Gleason grade may provide a potentially novel therapeutic target for prostate cancer.

Over the past decade, a limited number of studies have examined the effects of cannabinoids on prostate cancer cells. Table 1 summarizes the effects of cannabinoids on cell proliferation, migration and invasion. Most of these studies have focused on the use of the human prostate epithelial cells PC3, DU145 and LNCaP, which have greatly contributed to our collective understanding of the disease. In 2012, Nithipatikom et al. [23], reported a significant decrease in cell migration in PC3 cells upon treatment with 500nM of WIN55212-2. It was reported that treatment with the CB₁ antagonist AM251 reversed the anti-migratory effects of WIN55212-2 in these cells. This indicates a role for the CB₁ receptor in responses related to cell migration and motility. In a detailed study by Morell et al. [24], treatment with 3μM WIN55212-2 inhibited the neuroendocrine differentiation of LNCaP cells, suggesting a potentially beneficial treatment option for targeting a much more aggressive and difficult to treat subtype of prostate cancer, whereby clusters of cells differentiate into a neuroendocrine-like phenotype. Additionally, inhibition of the enzyme involved in 2AG synthesis resulted in an increase in cell invasion, which was attenuated by exogenous 2AG in PCa cells, including PC3, DU145 and LNCaP [25].

Proliferation studies examining the anti-cancer potential of cannabinoids in prostate cancer cells PC3, DU145, and LNCaP have shown consistent results. Orellana et al. [26], reported a significantly decreased viability in prostate cells (including PC3, primary cultures of prostate cancer derived from human tissue and benign prostatic hyperplasia) when treated with 2.5μM, 5μM, and 10μM of methanandamide, AEA, or 2AG. The effects were more pronounced in PC3 cells compared to both primary cultures. Additionally, other investigators [27] found that both methanandamide and JWH-015 caused a dose dependent decrease in cell viability at doses over 5μM. Subsequent analysis showed consistent results when DU145 and LNCaP cells were used, although a slightly weaker effect was observed in LNCaP. LNCaP cells treated with WIN55, 212-2 and CBD achieved a 50% reduction in growth at concentrations of 5μM and 10μM,

respectively [28]. In contrast, De Petrocellis et al. [29], observed a 50% growth inhibition in LNCaP cells at a concentration of 25μM CBD, however, this was not achieved in all the non-THC cannabinoids tested in DU145 and LNCaP cells. The above studies demonstrate that cannabinoids induce an inhibitory effect on the growth of prostate cancer cells. However, it is evident that follow up studies are warranted to investigate the pharmacological profiles of these compounds and to provide a better understanding of mechanisms that are involved in their antiproliferative or apoptotic effects.

In vivo studies using cannabinoids in prostate cancer animal models have been limited, and are usually restricted to LNCaP xenografts due to reduced efficacy of PC3 or DU145 cells. The administration of CBD by intraperitoneal (i.p) injection reduced tumor size in LNCaP xenografts, with CBD significantly enhancing the tumor suppressive effects of bicalutamide in these animal models. In contrast, CBD was unable to suppress tumor growth in DU145 xenografts, nonetheless potentiated the effects of docetaxel [29]. Morales et al. [30], showed almost complete inhibition of LNCaP tumor growth in mice treated with daily i.p administration of the SC quinine. However, subsequent analysis with PC3 xenografts demonstrated only 40% growth inhibition. Other investigators [27] report a 45% reduction in tumor size of PC3 xenografts after daily treatment with JWH-015; however, these studies did not report antitumoral properties of the cannabinoid in other xenograft models. None of these studies reported a potential mechanism of action based on *in vivo* results, thus additional studies are warranted to investigate cannabinoids in metastatic PCa animal models, including the Transgenic Adenocarcinoma Model of the Mouse Prostate (TRAMP), or the *Lady* Transgenic (12-T10/ 12T7) model. This will allow researchers to further elucidate appropriate dosage and time for optimal treatment intervention. Furthermore, studies exploring the combination of cannabinoids with chemotherapy is necessary to investigate potential adverse effects of multi-drug treatment and the molecular cross links between drugs.

Molecular targets mediated by cannabinoids

Many investigators have attempted to elucidate the molecular mechanisms through which cannabinoids alter tumorigenesis. Figure 1 depicts a simplified diagram outlining three main signaling pathways associated with cannabinoid receptor activation. The following sections will explore proposed mechanisms that have been brought to light, including those related to endoplasmic reticulum stress, oxidative stress, and Rho GTPase signalling.

Endoplasmic reticulum stress related pathway

The endoplasmic reticulum (ER) is an organelle recognized for its role in the synthesis, folding and modification of secreted, membrane-bound and organelle-targeted proteins [31]. Several factors are essential for optimum protein folding, including intraluminal calcium concentrations, ATP availability and an oxidizing environment for disulphide-bond formation. If these factors become disrupted, the ER can easily detect this stress [32]. A variety of physiological and pathological conditions such as calcium depletion, viral infections, and exposure to anticancer agents may cause an imbalance between ER protein folding load and capacity, leading to an accumulation and aggregation of unfolded proteins in the ER lumen, a condition referred to as

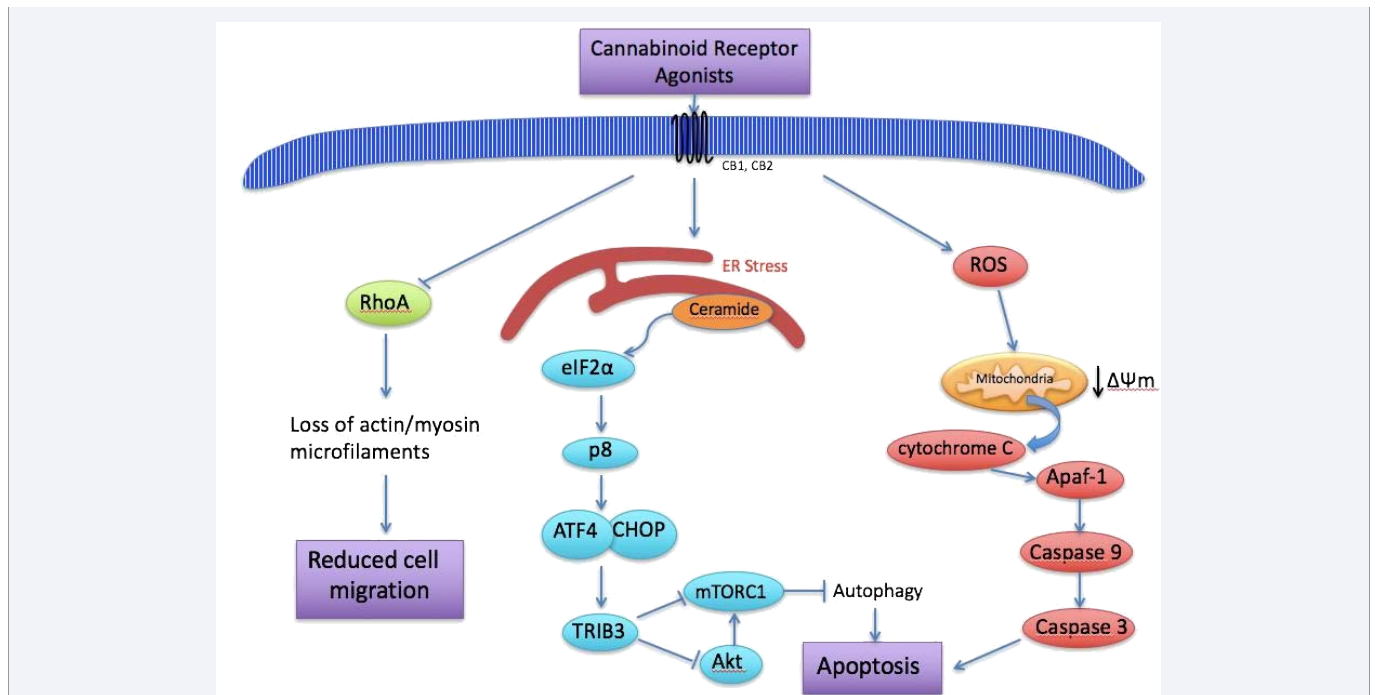


Figure 1 A simplified diagram that depicts signaling pathways associated with cannabinoid receptor activation induced by its agonists in prostate cancer. Upon receptor binding, cannabinoids inhibit cell migration through inhibition of RhoA GTPase. Accumulation of ceramide promotes endoplasmic reticulum stress, leading to an upregulation of p8, and subsequent induction of apoptosis. Cannabinoid receptor agonists also activate reactive oxygen species (ROS) generation leading to subsequent activation of the caspase cascade. The proposed mechanisms are based on available literature and are cell-specific, and may not be triggered simultaneously.

Table 1: Effects of cannabinoids at micromolar concentrations upon cell viability, migration, and invasion in human immortalized prostate cancer cells activation of mTOR and inhibition of AMPK Role of CB₂ implicated but not fully elucidated.

Cell Lines	Cannabinoid	Anticancer Effect	Mechanism of Action	Reference
PC3 prostate cancer cells	WIN55212-2	Decrease in cell viability motility	Activation of CB ₁ results in repression of RhoA activity (suppression of cell migration)	23
PC3, DU145, LNCaP prostate cancer cells	JWH-015 MET	Decrease in cell viability IN VIVO: Reduction in tumor growth	CB ₂ activation by JWH-015 inhibits Akt-mTOR pathway and activates eIF2α (induction of ER stress- proapoptotic effect)	27
PC3, DU145 prostate cancer cells	2AG	Inhibition of invasion	2AG activates CB ₁ receptor, inhibits adenylyl cyclase and decreases activity of PKA (inhibition of invasion)	25
PC3, and primary cultures of prostate cancer and benign prostatic hyperplasia tissue	AEA, 2-AG, MET	Decrease in viability Increase in apoptosis	Activation of CB ₁ receptor results in activation of apoptotic pathway without modification in cell cycle or necrosis Endocannabinoids modulate AKT and ERK	26

			pathways	
LNCaP prostate cancer cells	WIN, CBD	Inhibition of proliferation	WIN and CBD activate PARP cleavage and induce apoptosis WIN effects CB receptor independent and CBD CB receptor dependent	28
DU145 and LNCaP prostate cancer cells	CBD	Inhibition of viability	CBD induces ER stress and production of ROS	29
LNCaP prostate cancer cells	WIN	Inhibition of neuroendocrine differentiation	Inhibition of PI3K/Akt pathway results in	24

Abbreviations: CB: Cannabinoid Receptor; MET: Methanandamide; eIF2 α : Eukaryotic Initiation Factor 2 Alpha; mTOR: Mammalian Target of Rapamycin; ER: Endoplasmic Reticulum; 2AG: 2-arachidonoylglycerol; PKA: Protein Kinase A; AEA: Anandamide; ERK: Extracellular Signal-Regulated Kinases; WIN: WIN55,212,2; CBD: Cannabidiol; PARP: Poly (ADP-ribose) Polymerase; ROS: Reactive Oxygen Species; AMPK: 5' Adenosine Monophosphate-Activated Protein Kinase

ER stress [33]. To protect against the deleterious effects of ER stress, cells have evolved strategies that are collectively referred to as the unfolded protein response (UPR), in which protein translation and gene transcription are temporarily shut down. The UPR is considered a pro-survival response initiated to reduce the accumulation of unfolded proteins, thereby restoring normal ER functioning [34]. However, if this transcriptional programme fails to re-establish, persistent ER stress can cause a switch to a pro-apoptotic response. Over the past decade, it has been reported that cannabinoids exert their anticancer effects through activation of apoptosis. It is postulated that these pathways result in the production of ceramide, which may induce ER stress and initiate intrinsic apoptosis. The inability to return to ER homeostasis may result in cell death by a mechanism involving mammalian target of rapamycin (mTOR) pathway inhibition, and subsequently, autophagy [35-39]. Olea-Herrero et al. [27], have provided support for this notion, whereby CB₂ receptor activation by the SC JWH-015 induces synthesis of ceramide in PC3 cells, inhibiting the Akt-mTOR pathway and activating initiation factors involved in autophagy regulation and the ER stress response. This effect was dependent on CB₂ activation, as combined treatment with CB₂ antagonist SR144528 resulted in the prevention of cell death and a decrease in the synthesis of intracellular ceramide.

Conversely, increases in ceramide levels and ER stress may trigger activation of the caspase cascade leading to apoptosis. Orellana et al. [26], demonstrated an increase in the levels of active caspase-3, a decrease in the expression levels of Bcl-2, and a decrease in the expression of Akt after treatment with the endocannabinoids 2-AG, AEA and methanandamide in primary cultures of prostate cancer. This study suggests an inhibition in Akt may contribute to the activation of anti-proliferative pathways. Unlike Olea-Herrero et al., these effects were CB₁ dependent, as combination treatment with the CB₁ antagonist SR141716 prevented apoptosis in these cells. Future studies are warranted to clarify the role of the cannabinoid receptors in the activation of ER stress related pathways and to elucidate the link between CB receptors and downstream targets in the ER stress response.

Oxidative stress

Reactive oxygen species (ROS) are generated during every day metabolic processes in normal cells and play a vital role in cell signalling. However, excessive production of ROS or an inadequate antioxidant defence system may lead to a phenomenon known as oxidative stress, which has been associated with the initiation and development of a variety of cancers, including prostate cancer [4-,41]. Supporting evidence has suggested that increasing ROS production in prostate cancer cells are associated with aggressive phenotype, hence, targeting ROS production might offer a potential approach in preventing cancer development [42]. Paradoxically, oxidative stress occurring at the intracellular level can have chemopreventive effects and may be used as an anticancer agent to induce apoptosis in malignant cells. Various studies have reported that chemopreventive agents work in some part by generating ROS and disrupting redox homeostasis [43,44]. In this scenario, ROS act as secondary messengers that influence mitochondrial function, mediates the elevation of intracellular calcium and activates the caspase cascade. ROS production may induce pro-apoptotic signals leading to the release of proteins from the mitochondrial intermembrane space into the cytosol, thereby promoting apoptosis [45,46]. Several studies have demonstrated that cannabinoids induce apoptosis in cancer cells through the production of ROS. De Petrocellis et al. [29], report an elevation of intracellular calcium and activation of ROS production in LNCaP cells after treatment with CBD. This would suggest that ER stress and oxidative stress are contributing factors in the pro-apoptotic effect of CBD. These results were also seen in non-AR expressing cells, DU145 and PC3, indicating that CBD increases ER and oxidative stress with no involvement of AR and p53 status. It is speculated that ROS is necessary for the increase in the AMP/ATP ratio, which subsequently mediates the activation of AMPK by cannabinoids and leads to cell death. Research proposes that ROS production by cannabinoids activates a positive feedback loop. In this positive feedback loop, electron transport chain inhibition leads to NADH accumulation and the subsequent inhibition of oxidative phosphorylation, amplifying the production of ROS [47]. Alternatively, ROS production may trigger the release of pro-

apoptotic proteins such as cytochrome c, caspase-9, apoptosis inducing factor, and Smac/DIABLO from the inner mitochondrial membrane space into the cytosol. Researchers showed that AEA induced cell death through a pathway involving mitochondrial uncoupling and cytochrome c release, which may be mediated by oxidative stress and ROS production through a TRP-dependent mechanism [48,49]. Similarly, Massi et al. [50], report an induction of ROS production after CBD exposure in human glioma cells, with a time course preceding caspase-8 and -9 activations. Despite increasing evidence associating cannabinoid treatment to increased ROS production and oxidative stress, conflicting findings regarding the benefit and/or harm of ROS production cannot be ignored. Thus, an in-depth analysis of these apoptotic pathways is warranted to develop a deeper understanding of the future use of cannabinoids as anticancer treatment modalities.

Rho GTPase Signalling

Cell migration is an integral process that controls inflammation and morphogenesis. Once deregulated, cell migration is associated with many disease states, including autoimmune syndromes, chronic inflammation, and cancer [51]. A variety of intracellular signalling molecules have been implicated in cell migration and invasion, including phospholipases, Tyr kinases, lipid kinases, Ser/Thr, and MAPK cascades. Of these, the protein family most pivotal to the regulation of cell migration and invasion is the Rho GTPases. The most well studied and highly conserved Rho GTPases include Rho, Rac, and Cdc42. Rho family GTPases regulate cell migration through the assembly of actin/myosin filaments, cell adhesion and spreading, and the establishment of cell polarity [52-54]. Under pathological conditions such as tumor invasion and metastasis, cells become detached from the primary tumor and enzymatically degrade the extracellular matrix or basement membrane of tissues to become established in a new location. Critical downstream components in Rho-GTPase signalling and actin binding proteins have been linked to metastasis *in vivo*. In prostate carcinoma cells, activity of RhoA is amplified and corresponds to an increase in cell migration and invasion. The amplification in RhoA is induced by the stimulation of multiple G protein coupled receptors (GPCR) for thrombin and thromboxane A2 [55,56]. In view of this, studies have explored the ability of cannabinoid receptor activation to repress RhoA activity, thereby providing a novel mechanism to diminish migration and invasion of aggressive prostate carcinoma cells. Nithipatikom K et al. [23], have reported that activation of CB₁ with endogenous agonists AEA and 2-AG results in the suppression of RhoA activity in PCa cells, contributing to the suppression of cell migration. The study details a loss of RhoA activity accompanied by the loss of actin/myosin microfilaments, reduced cell migration, and decreased cell adhesion. Similarly, studies [57,58] using highly aggressive breast cancer cells MDA-MB-231 reported CB₁ mediated inhibition in GTPase activity of RhoA. This suggests that the inhibition of RhoA by cannabinoids mitigate Rho's ability to promote invasion by causing a disruption in RhoA membrane localization, necessary for its interaction with several signalling components. Other studies [25,59] have shown a CB₁ dependent inhibition of adenylyl cyclase and protein kinase A, resulting in a reduction of RhoA activity, and subsequent decreases in prostate and breast cancer cell invasion. Despite conclusive evidence regarding CB receptor mediated reductions

in RhoA activity, a deeper understanding of signalling events that cause CB receptor dependent alterations in Rho GTPase activity is warranted. This will help us understand how RhoA is targeted by cannabinoid receptor stimulation and whether this pathway is responsible for cannabinoid induced inhibition of cell migration and invasion.

CONCLUSIONS AND FUTURE DIRECTIONS

Over the past few decades, a large volume of research on the therapeutic potential of cannabinoids have accumulated in the field of cancer for both its palliative and anticancer properties. While a majority of mechanistic evidence is still lacking, particularly in the field of prostate cancer, it is difficult to ignore the accumulating evidence implicating a role for cannabinoids in cancer cell death, the prevention of metastasis, and the mitigation of tumor growth [60]. Nevertheless, it is necessary to complete studies in all PCa and healthy cells to verify the specific anticancer properties of cannabinoids, including the most widely available phytocannabinoids, endocannabinoids, and synthetic cannabinoids. Additional studies should be completed *in vivo* using xenograft and/or transgenic models of PCa to investigate the role of cannabinoids in tumor growth and metastasis, as well as to determine appropriate doses and optimal intervention times. Little information is available on the pharmacokinetics, metabolism and route of administration of cannabinoids both in animals and human, making the transition towards clinical studies challenging. Hence, future studies investigating various components of the endocannabinoid system and their relation to cancer tissue is warranted. Studies should explore the pharmacokinetics of drug administration, and investigate the expression of cannabinoid receptors in metastatic tissue to better understand the role of the endocannabinoid system in disease outcome and progression.

It is important to note that cannabinoids are generally well-tolerated compounds. For example, non-psychoactive substances such as CBD can be administered up to 1500 mg/day without the production of adverse side effects [61]. This low toxicity and preference for acting on cancerous cells as opposed to healthy cells provides an advantage over more conventional anticancer treatments, which are non-selective to malignant cells and are associated with a variety of side effects. Although cannabinoids are effective in micromolar concentrations as opposed to chemotherapeutic agents such as docetaxel, which require nanomolar dosages, their safe toxicological profile place them as promising candidates for use in combination therapy. Further research will be required to have a better understanding of the molecular cross talk between cannabinoids and chemotherapeutic agents. One such approach is to discover biomarkers in tumor biopsies or in serum that might contain circulating cancer cells following cannabinoid therapy. This will uncover drug pharmacodynamics and can be used as a predictive marker for PCa subtypes most sensitive to cannabinoid-based therapy.

This review provides support for the continued investigation of cannabinoids as an anticancer agent. Approaches that target the endocannabinoid system may offer new opportunities for the treatment and prevention of prostate cancer. Accumulating evidence supports the involvement of endocannabinoids in

cancer regulation, however most endocannabinoids are rapidly metabolised *in vivo* and thus would not be feasible for patient use as a single agent treatment. In addition, numerous studies have highlighted the role of synthetic and phytocannabinoids in cancer growth and metastasis. However, due to their high potency and binding affinity for the CB receptors, their use has been associated with significant psychoactive side effects. Although a majority of literature supports future therapeutic uses of cannabinoids in cancer treatment, it is crucial to determine the role of the CB receptors in mediating the effects of cannabinoids in cancer cells.

This may allow for approaches that reduce the psychoactive effects while maintaining its therapeutic benefits and is necessary for a successful introduction into conventional prostate cancer treatment.

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