Cannabinoid Signaling in Cancer

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Subhadip Das, Kirti Kaul, Sanjay Mishra, Manish Charan, and Ramesh K. Ganju

Abstract

The family of chemical structures that interact with a cannabinoid receptor are broadly termed cannabinoids. Traditionally known for their psychotropic effects and their use as palliative medicine in cancer, cannabinoids are very versatile and are known to interact with several orphan receptors besides cannabinoid receptors (CBR) in the body. Recent studies have shown that several key pathways involved in cell growth, differentiation and, even metabolism and apoptosis crosstalk with cannabinoid signaling. Several of these pathways including AKT, EGFR, and mTOR are known to contribute to tumor development and metastasis, and cannabinoids may reverse their effects, thereby by inducing apoptosis, autophagy and modulating the immune system. In this book chapter, we explore how cannabinoids regulate diverse signaling mechanisms in cancer and immune cells within the tumor microenvironment and whether they impart a therapeutic effect. We also provide some important insight into the role of cannabinoids in cellular and whole body metabolism in the context of tumor inhibition. Finally, we highlight recent and ongoing clinical trials that include cannabinoids as a therapeutic strategy and several combinational approaches towards novel therapeutic opportunities in several invasive cancer conditions.

Keywords

Cannabinoids · Tumor microenvironment · Signaling · Metabolism

Abbreviations

2-AG	2-arachindonoylglycerol
AEA	anandamide
AMPK	5′ AMP-activated protein kinase
CBD	cannabinoids
CBR	cannabinoid receptor
COX-2	cyclooxygenase-2
CXCL	C-X-C motif chemokine ligand
EGFR	epidermal growth factor receptor
EMT	epithelial to mesenchymal transition
E:R	endoplasmic reticulum
mTOR	mammalian target of rapamycin

The authors Subhadip Das and Kirti Kaul have equally contributed to this chapter.

S. Das · K. Kaul · S. Mishra · M. Charan
R. K. Ganju (✉)
Department of Pathology, The Ohio State University, Columbus, OH, USA

Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA
e-mail: Ramesh.ganju@osumc.edu

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PPAR	peroxisome proliferator-activated receptors
ROS	reactive oxygen species
TAMs	tumor associated macrophages
THC	Δ9-tetrahydrocannabinol
TME	tumor microenvironment
TRPV2	transient receptor potential cation channel subfamily V member 2.

4.1 Introduction

Cannabinoids have typically been assumed to originate from the plant *Cannabis*, however, broadly speaking cannabinoids are the group of chemical structures that mainly act in the body through cannabinoid receptors (CB); CB1 (Central receptor) and CB2 (Peripheral receptor) [1, 2]. They can be divided into different groups based on their source of origin as plant derived cannabinoids (phytocannabinoids), endogenously produced cannabinoids (endocannabinoids) and chemically produced synthetic cannabinoids. They all represent a broad range of ligands that interact with the CB receptors termed cannabinoids.

Amongst the several phytocannabinoids, Δ9-tetrahydrocannabinol (THC) is the main psychoactive compound. These compounds are responsible for many physiological effects such as euphoria, pain relief and anti-inflammatory activities [3].

Endogenous ligands like anandamide (AEA) and arachindonoylglycerol (2-AG) interact with CB as part of the endocannabinoid system [4]. Majority of the CB are expressed in neural tissues as CB1 receptor, and are known to modulate the central nervous system. CB2 receptors are predominantly expressed in immune cells and thus can modulate both the innate and adaptive immune systems [5–8] Interestingly, cannabinoids bind not only to classical receptors (CB1 and CB2), but also to certain orphan receptors and ion channels like transient receptor potential vanilloid-2 (TRVP2) and peroxisome proliferator-activated receptors (PPAR) [9] (Table 4.1).

4.2 Cannabinoids and Cancer Signaling

Several studies have suggested that cannabidiol and THC directly inhibit cancer cells growth by activation of diverse signaling pathways associated with apoptosis, proliferation, angiogenesis and metastasis [10, 11]. A schematic representation of these pathways is presented in Fig. 4.1 [12]. Previously, it has been reported that THC mediates its pro-apoptotic effect in tumor cells by increased synthesis of the proapoptotic sphingolipid ceramides [13]. In glioma cells, ceramide-dependent upregulation of the stress protein p8 induced apoptosis via the upregulation of the endoplasmic reticulum (ER) stress related gene Activating Transcription Factor *4 (*ATF-4), C/EBP homologous protein (CHOP) and Tribbles homolog 3 (TRB3) [14]. It was also found the ceramide can induce apoptosis in leukemic cells by regulation of p38 MAPK signaling. Experimental studies also revealed that THC causes apoptosis in leukemia T cells by down-regulation of Raf-1/mitogen-activated protein kinase/ERK kinase pathway and thus, leads to translocation of BCL2 Associated Agonist of Cell Death (BAD) to mitochondria [15]. On the other hand, it can activate apoptosis in colorectal cancer cells by inhibition of RAS-MAPK/ERK and PI3K-AKT survival signaling cascades accompanied by activation of the pro-apoptotic BAD [16].

Most interestingly, THC promotes autophagy mediated apoptosis by inducing ceramide accumulation via Tribbles homolog 3 dependent inhibition of the AKT/mTORC1 complex axis in human glioma [4] and in hepatocellular carcinoma [17] cells. The combined administration of THC and temozolomide was also found to exert a strong anti-tumoral effect *in-vivo* in in glioma mouse model [18]. THC treatment was also reported to inhibit the proliferation of breast cancer cells by activating the CB2 receptors with subsequent arrest of cell cycle in G2-M phase via downregulation of Cyclin-Dependent Kinase 1 (CDC2) protein [19] or modulation of JunD (a member of the AP-1 transcription factor family)

Table 4.1 Role of cannabinoids in different physiological processes

Cannabinoids	Target receptor	Effect
Anandamide (AEA)	CB1	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor
2-arachidonoyl-glycerol (2-AG)	CB1/CB2 agonist	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor
Palmitoyl-ethanolamide (PEA)	CB2 agonist	Neuromodulatory and immunomodulatory
Docosatetraenyl ethanolamide	CB1 agonist	Neuromodulatory and immunomodulatory
Homo-γ-linoenylethanolamide	CB1 agonist	Neuromodulatory and immunomodulatory
Oleamide	CB1 agonist	Neuromodulatory and immunomodulatory
Δ9-tetrahydrocannabinol (Δ9-THC)	CB1/CB2 agonist	Analgesic, antiemetic, appetite stimulant tumour growth inhibitor
Δ8-tetrahydrocannabinol (Δ8-THC)	CB1/CB2 agonist	Anti-tumor agent, inhibitors of mitochondrial O_2 consumption in human sperm, antiemetic, appetite stimulant
Cannabidiol (CBD)	CB1 agonist	Anti-tumor agent, attenuate catalepsy, immunosuppressive, inflammatory or anti-inflammatory agent (depends upon used concentration of drug), antipsychotics
Cannabigerol (CBG)	CB1/CB2 agonist	multiple sclerosis, antiemetic, anti-inflammatory agent, treatment for neurological disorder
Cannabichromene (CBC)	CB2 selective agonist	anti-inflammatory agent, treatment for neurological disorder, hypomotility, antinociception, catalepsy, and hypothermia
Tetrahydrocannabivarin (THCV)	CB1 antagonist and partial CB2 agonist	Hepatic ischaemia, anti-inflammatory
HU-210	CB1/CB2 Nonselective agonist	Analgesic, multiple sclerosis, neuroprotective
CP-55,940	CB1/CB2 Nonselective agonist	Anti-cancer agent, Analgesic, antiemetic, appetite stimulant
R-(+)-WIN 55,212-2	CB1/CB2 Nonselective agonist	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor, multiple sclerosis
JWH-015	CB2 selective agonist	Anti-tumor, anti-inflammatory, antiemetic
JWH-133	CB2 selective agonist	Neurological disorders, Anti-cancer
JWH-139	CB2 selective agonist	Analgesic, antiemetic, appetite stimulant tumour growth inhibitor
HU-308	CB2 selective agonist	Tumour growth inhibitor (in glioma, skin carcinoma, lymphoma
CP55940	CB/CB2 agonist	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor, multiple sclerosis
R-(+)-methanandamide	CB1 agonist	Analgesic, antiemetic, appetite stimulant tumour growth inhibitor
AM251	CB1antagonist	Metabolic syndrome
AM281	CB1antagonist	Improves recognition loss induced by naloxone in morphine withdrawal mice, various pharmacological property

[20] It also upregulated several PPAR dependent signaling pathways in cancer cells [21].

Additionally, further study confirmed that cannabidiol inhibited cancer cell viability and proliferation, which was reversed *in-vitro* in the presence of blockers of either CB2, Transient Receptor Potential Vanilloid 1 (TRPV1) or melastatin-related transient receptor potential (TRPM), cyclooxygenase-2 (COX-2) or PPAR and in tumor derived primary culture from a patient with non-small cell lung cancer in presence of PPAR antagonists [22, 23]. Our research group demonstrated that cannabinoid mediate its anti-proliferative effects in highly aggressive

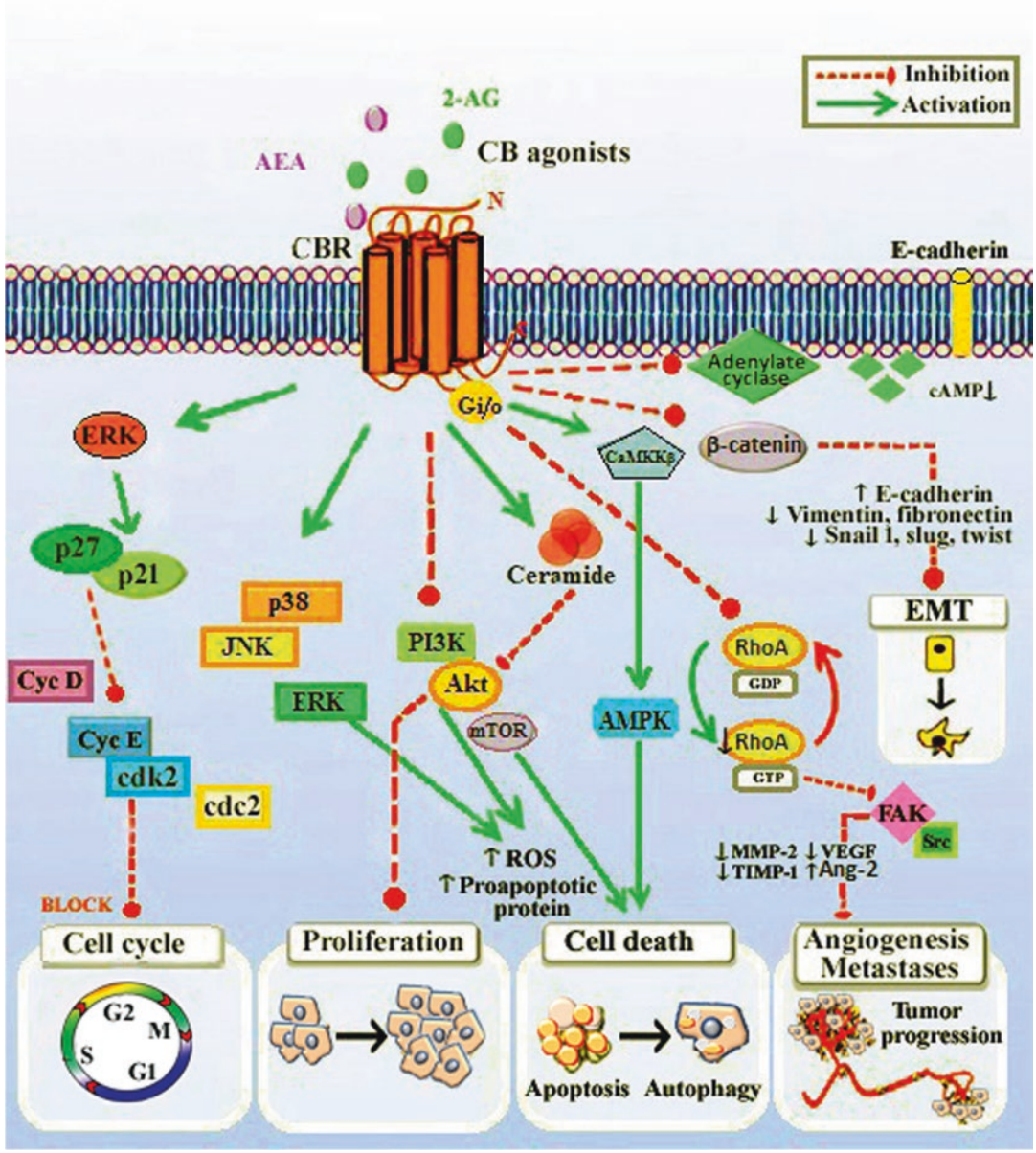

Fig. 4.1 Schematic representation of the main signaling cascades highlighting the downstream CB receptor activation by cannabinoids which impact all the hallmark processes of cancer such as proliferation, apoptosis, migration, invasion, angiogenesis and EMT (epithelial-mesenchymal transition)

human breast cancer cells in part, by inhibition of epidermal growth factor (EGF), NF-kB, extracellular signal–regulated kinases/AKT (ERK/AKT) and matrix metalloproteinase 2 and 9 signaling pathways [24]. Cannabinoid also reduces angiogenesis in both tumor and endothelial cells. Experimental evidence suggested that cannabinoid treatment also suppresses the expressions of pro-angiogenic gene (COX-2) and decreases the secretion of prostaglandin E2 (PGE2) [25]. Furthermore, it is reported that anandamide showed decreased phosphorylation of focal adhesion-associated protein kinases, which are

components of cell adhesion machinery, and influence their migration [26].

Furthermore, the treatment of THC also inhibits the growth of Lewis lung adenocarcinoma via inhibition of DNA synthesis [27]. It has also been found that THC suppresses the growth and metastasis of A549 and SW-1573 (human lung cancer cell lines) both *in-vitro* and *in-vivo* by inhibition of epidermal growth factor-induced phosphorylation of ERK1/2, c-Jun-NH2-kinase1/2 and Akt [27, 28]. Recently, research studies from our group also revealed that CB2-specific synthetic cannabinoids, JWH-015 inhibits CXCL-12 induced migration and invasion by suppressing the phosphorylation of ERK and C-X-C chemokine receptor type 4 (CXCR4) polymerization [29]. It was also reported that the treatment of cannabinoids induces apoptosis in different malignant immune cells (Jurkat and EL-4) in lymphomas and leukemia's [30] via mitochondria mediated ROS pathway and activation of different caspases [31].

4.3 Cannabinoids and the Immune System

Presently, many advanced therapeutic approaches have been developed to treat different cancers which mainly include surgery, radiation and chemotherapy, endocrine therapy, or targeted therapy. Although, these therapies have decreased breast cancer specific mortality, they have also shown dramatic failures due to the emergence of drug resistance, relapse, multi-organ metastasis and subsequently death [32, 33]. Recently, it has been reported that the tumor microenvironment (TME) plays an essential role in regulating the stemness and drug resistance of cancer cells. TME play important roles in tumor initiation, development, invasion, and metastasis. TME is basically comprised of cancer cells, endothelial cells, fibroblasts and different types of immune cells known as tumor associated macrophages (TAMs).

TAMS have been shown to secrete different types of growth factors which can regulate TME and thus support cancer growth and subsequent metastasis [34]. Moreover, it has also been reported that M2 macrophages, which can secrete a diverse array of essential growth factors, can promote invasion and metastasis of cancer cells into multiple organs [34]. Recently, research findings have shown that the *in-vivo* treatment of Cannabidiol inhibits the recruitment of total macrophages and especially, M2 macrophage populations in tumor stroma as well as in lung metastatic nodules [24]. In this study, Zhu et al. demonstrated that that the *in-vitro* treatment of 4T1.2 cells with cannabinoid inhibited the secretion of specific cytokines such as CCL3 and GM-CSF in its condition medium (CM) as compared to CM of vehicle control. The CM harvested from cannabinoid treated 4T1.2 cells also significantly reduced the migration of mouse monocytic cells, RAW 264.7, comparatively to CM collected from vehicle control [24].

Furthermore, research findings also showed that the cannabinoid treatment inhibited the M2 macrophages induced epithelial mesenchymal transition (EMT) in non-small cell lung cancer (NSCLC) cells via downregulation of EGFR signaling cascade [35–37]. It has been reported that TAM can secrete EGF like ligands which can activate EFGR pathway and thus can cause increased EMT in cancer cells [38]. In addition, it has been shown that the treatment of JWH-015 inhibits the EMT induction by suppressing the activation of EGFR signaling in NSCLC cells both *in-vitro* and *in-vivo* systems. The treatment of JHW-015 also reduced the expression of proliferative marker (Ki67), angiogenic marker (CD-31), EMT markers (N-Cadherin, Snail and Slug) and also inhibited the infiltration of CD11b/F4/80/CD206 M2 macrophages into tumor. Investigation of these interactions and signaling has led to novel insights in the cannabinoids-mediated modulation of TME in cancer [39].

It has also been reported that the treatment of cannabinoids induces the conversion of T helper 1 cell (Th1) to T helper 2 cell (Th2) subpopulations by activating the expression of interleukin (IL), IL-10, and TGF-β and also decreases the production of TH1 cytokines (IL-2, IL-12 and Interferon-γ) [40] [41]. On the other hand, IL-10 and TGF-β play significant roles in mediating the

THC induced suppression of anti-tumor immunity, and abrogation of either cytokine alone is sufficient to reverse the detrimental effect of THC. The study suggests that THC promotes tumorigenicity and limits immunogenicity *in-vivo* by upregulating the potent immune inhibitory cytokines [42].

THC has also been reported to modulate the activity of different immune cells such as macrophages, NK cells and T lymphocytes. THC and other cannabinoid agonists may exert their immune modulating effects through the disruption of Th1 to Th2 conversion [43]. THC mediates these effects by inhibiting the production of type-1 cytokines and promoting type-2 cytokine production by lymphocytes [44]). The synthetic analogues of cannabinoid were also reported to suppress the proliferation of T cells by inhibiting the production of IFN-γ. So overall, several studies show that cannabinoid and its different synthetic analogues can modulate host immunity and thus, it can regulate tumor growth and metastasis in different human malignancies [45].

4.4 Cannabinoids in Cellular Metabolism

Aberration of cellular metabolism is a hallmark feature of solid tumors as well as leukemic cancers [46–48]. For several decades, cancer associated metabolism has been defined in context of the Warburg effect, which suggests that highly proliferative cancer cells are entirely dependent on glycolysis rather than the mitochondria driven oxidative phosphorylation for their energetics. Since Warburg's initial observations, research has questioned the dogma of the Warburg effect and helped to establish the significant contributions of metabolic reprogramming in mitochondrial function, and cellular energetics in cancer cell survival, metastasis, and even drug resistance [49–51]. Moreover, recent experimental and epidemiological research has also implicated whole body metabolism and changes induced by factors such as high fat diet, particularly obesity, in the development of a pro-tumor microenvironment [52] [53]. This has led to a greater interest in targeting cellular metabolic pathways, such as the 5′ AMP-activated protein kinase (AMPK), protein kinase C (PKC), and mammalian target of rapamycin (mTOR) pathways [54–56], Although PKC inhibitors have been successfully tested in the experimental setting, the efficacy of these inhibitors as monotherapy against cancer has been limited to B-cell lymphoma malignancy [57] Similarly, targeting other individual pathways, including mTOR, AKT and AMPK have had limited success in eliciting anti-tumor activity. Another product of cellular metabolism, particularly in cancer cells is reactive oxygen species (ROS). ROS are thought to further contribute to inflammatory pathways and damage cellular macromolecules and nucleotides, particularly DNA, thus potentially perpetuating cancer survival and metastasis [58].

Meanwhile, growing body of research in cannabinoids indicates a close mechanistic link between cannabinoids and metabolism. Cannabinoids have primarily been investigated as palliative therapy for individuals with advanced cancer. In this section, we hope to provide an overview of current literature linking cannabinoids and their anti-tumor activity mediated through metabolism and metabolic pathways, thereby shedding light on the potential of cannabinoids as a therapy against cancer.

For several decades, the link between metabolic syndrome and obesity, and cancer has sparked interest in whole body metabolism in patients with cancer. Fatty acid oxidation by tumor cells is often linked with various cancers, including prostate cancer, breast cancer, pancreatic cancer, etc. [59–61]. Fatty acid oxidation, mediated through the mitochondria, is a highly energetic process linked with high ROS generation. Experimental studies have shown that an inhibition of mitochondrial metabolism and a switch to glycolytic energy generation in tumors is linked with better prognosis as well as drug response [62]. Mitochondrial uncoupling is also critically important in inducing programmed cell death, thus making this shift from mitochondrial respiration to glycolysis in tumor cells a key therapeutic target [63].

It has also been observed that patients with cancer undergo greater loss of lean mass rather than fat mass, which in turn is linked with poor outcome and quality of life. Lean mass loss in patients with cancer can be regulated by protein nutritional support, however, the course of therapies often leads to loss of appetite, which greatly impact nutrition in these patients [64]. This is partially benefitted by cannabinoids, as cannabinoids enhance appetite through Ghrelin receptor interaction. Ghrelin receptor, a receptor for the anabolic hormone Ghrelin, is expressed is all vital organs. It is known to modulate appetite, fat accumulation and energy expenditure. Moreover, the synthetic cannabinoids HU210, impacts cellular energy metabolism via Gherlin receptor interaction [65].

In spite of its central role in nutrient sensing and metabolic regulation, AMPK appears to have both pro-tumor and anti-tumor effects. On one hand, AMPK promotes this metabolic plasticity through promotion of fatty acid oxidation, while on the other hand AMPK is closely linked with tumor suppressors p53 and tuberous sclerosis complex (TSC2) [55]. While a thorough investigation of AMPK subunits and variants involved in various solid tumors and leukemia has never been performed, several studies indicate reduced AMPK activity in lung cancer, colorectal cancer, breast cancer, ovarian cancer, hepatic cancer, etc. [1, 60, 66–69]. Interestingly, AMPK activation in cancer models, including hepatoma, has been shown to inhibit PPAR-gamma and PCG-1alpha leading to a decrease in fatty acid oxidation [70] The effect of the mitochondrial inhibitor, metformin, in patients with breast cancer is also shown to be mediated by AMPK [71].

Although not as extensively investigated in different models and cancers, synthetic cannabinoids arachidonoyl cyclopropamide (APCA) and GW405833 have been shown to inhibit mitochondrial metabolism and induce AMPK-dependent autophagy in pancreatic cancer cells [72]. Cannabinoid receptor cross-talk with AMPK is well documented in several tissues and is linked with reduction in mitochondrial biogenesis, thus disrupting mitochondrial metabolism [73]. Another systemic effect of Cannabinoids on metabolism as well as their anti-tumor activity may be exerted through the insulin signaling pathway. The key factors downstream of insulin-insulin receptor interaction such as AKT, mitogen activated protein kinase kinase 1/2 (MEK1/2) and ERK are known to contribute to cell proliferation, motility, and cancer cell survival. Cannabinoids have been shown to induce hepatic insulin resistance and multiple studies report that Cannabinoids inhibit insulin receptor signaling in pancreatic beta cells showing direct interaction between the CB1 receptor and insulin signaling [74, 75]. This is an entirely new and therapeutically sound avenue to alter crucial cell survival pathways with minimal toxicity to healthy cells.

Cancer cells have high energy needs to maintain proliferation and migration. Cannabinoids are known to inhibit mitochondrial energetics leading to autophagy [76] In pancreatic cancer cells, in combination with Gemcitabine, APCA is known to induce ROS-mediated autophagy, once again suggesting the possible role of mitochondrial electron transport chain uncoupling in response to Cannabinoids, thus directly affecting cancer cell death [77].

Cannabinoid may also potentially induce anti-tumor activity via immune cell, particularly macrophages. It has been observed that the activation of CB1 by ACEA in macrophages, which modulates ROS production, is dependent on the phosphorylation of p38-mitogen-activated protein kinase (p38-MAPK). This is known to lead to tumor necrosis factor-α and monocyte chemoattractant protein-1 expression, thus enhancing a pro-inflammatory phenotype [78]. Nevertheless, the direct effects of Cannabinoids on macrophage phenotype and function have not been thoroughly tested.

Finally, it is important to note that in the present epidemic of metabolic diseases and obesity that drive various cancers, phytocannabinoids, particularly THC, act in a manner similar to metformin. Metformin is a mild inhibitor of complex 1 of the mitochondria, and therefore is thought to play an important role in metabolic reprogramming. While long term use of metformin has been linked with risk of cardiomyopathy, the effect of chronic use of phytocannabinoids and synthetic

cannabinoids on systemic health, while predictable based on several studies, may need to be performed specifically in the context of cancer survivor cohorts [79].

4.5 Recent Advances of Cannabinoids in Clinical Trials

The use and understanding of mechanisms of cannabinoids in context of tumors are almost completely limited to preclinical studies. Nevertheless, its lower toxicity led it to the first clinical application of THC on humans, conducted on nine terminal patients with recurrent glioblastoma and resistant to standard chemotherapy [85]. THC was administered intratumorally and dose was determined to be safe and without any psychoactive effects [80]. Furthermore, this study also confirmed the antiproliferative action and induction of apoptosis induced by THC, however, further studies are needed to determine the correct dosage or any potent systemic interaction [80]. There have been some clinical trials currently ongoing or recently completed using combinatorial treatments of nabiximols and temozolomide in patients with recurrent glioblastoma (NCT01812603, NCT01812616). Another clinical study was conducted using Cannabidoil as a single regimen on different solid tumor patients (NCT02255292). In addition, many recent clinical studies also underscore the promising therapeutic potential of one of the synthetic cannabinoids, dexanabinol, in patients with different solid tumors or brain cancer, compared with other healthy subjects (NCT01489826, NCT01654497, and NCT02054754).

4.6 Concluding Remarks

In summary, cannabinoid modulates the tumor growth and metastasis in different human malignancies by regulating different signaling cascades linked with proliferation, survival, angiogenesis and metastatic spread of cancer cells. It can also regulate the TME by regulating different types of immune cells associated with pro and anti-tumor immunity. Cannabinoid may induce ROS generation and lower mitochondrial activity in cancer cells, leading to autophagy and cell death. Cannabinoid also cross-talks with cellular metabolism via AMPK and mTOR, subsequently enhancing cancer cell death. Overall, application of cannabinoid will have high translational significance and impact for developing novel immune and metabolic-based therapies directed against different metastatic cancers with minimal to low side effects.

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