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Chapter Title	The Endocannabinoid System as a Target in Cancer: Status and Future Perspectives	AU1 AU2
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Abstract	The endocannabinoid system (ECS) represents a complex network of different molecules as cannabinoid receptors, endocannabinoid ligands, and the enzymatic machinery that drives their metabolism, as well as cells and pathways that use endocannabinoid signaling. It is important for the regulation of most vital biochemical processes contributing to and overall homeostasis state. As such, it is ambiguously implicated in both the development of cancer and its suppression, as well as its progression and interaction with current anti-cancer therapeutics. This work will review the main ECS components and their discovery, structure, pharmacological properties, and significance in various physiological and pathological states and focus on the current burden of evidence available from open-access databases, experimental data, and expert reviews which offer future directions for its use in the oncological setting. The vast potential of the translationally significant information of the so-called endocannabinoidome is currently being explored in many ongoing clinically oriented research studies as well as clinical trials. Previously acquired pharmacological data from its historical application in pain alleviation and as a general palliative agent in oncology will be useful for drug repurposing scenarios, aiming to speed up its possible clinical applications, after decades of carrying the stigma of an ethically and legally compromised target.

Keywords (separated by “-”)	Anti-cancer treatment - Endocannabinoid system - Cannabinoid receptor - Receptor heteromer

The Endocannabinoid System as a Target in Cancer: Status and Future Perspectives

AU1
AU2

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Contents

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N. Rezaei (ed.), *Handbook of Cancer and Immunology*,
https://doi.org/10.1007/978-3-030-80962-1_276-1

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Introduction

The constituent parts of the endocannabinoid system (ECS) represent an important cellular homeostatic network involved in putative signaling pathways in many cell types and tissues. Its significance has been reported in various physiological and pathological processes, as lipid and carbohydrate metabolism, food intake regulation, inflammation and neurodegeneration, respiratory health and diseases, and cancer. ECS components as cannabinoid receptors, endocannabinoid ligands, plant-derived and synthetic cannabinoids, and the enzymatic machinery involved in the metabolism of endocannabinoids have been explored as potential anti-cancer targets, as well as factors involved in cancer initiation, progression, metastatic spread, and prognosis. The ability of cannabinoid receptors to form functional heteromers with different biochemical properties than the constituent monomers

adds to the pharmacological diversity of possible outcomes upon ECS stimulation. The involvement of non-canonical pathways, which employ axes of secondary messengers as the alteration in intracellular Ca^{2+} levels and activation of protein kinases, contributes to the plasticity of expected ECS-induced effects, making the ECS a pharmacological treasure isle both for cancer research and beyond. As the action of cannabinoids has been linked to cancer cell death, autophagy, alterations of cell cycle, regulation of angiogenesis, and metastatic spread, as well as interaction with the tumor microenvironment, the specific effect they might induce depends on the specific tumor subtype in question, tumor stage, and pre-treatment modalities. Understanding the key elements and mechanisms of ECS's involvement in these essential biochemical signaling axes and their interactions is of utmost importance for progressing from preclinical to clinical studies and beyond. The ECS pharmacological potential is vast and attractive but must be accompanied by rigorous research and independent validation of obtained data. Researchers involved in deciphering these tasks carry an interesting yet responsible burden of weight toward unbiased planning and precise analysis of results, leading to a most useful output for the ECS – exploring its full milieu of anti-cancer activity while not embarking on psychotropic or other adverse events. For this to be achieved, the ECS components and their modalities of action must be thoroughly reviewed and reassessed in each setting.

The Components of the Endocannabinoid System

The extended ECS is a complex network of cannabinoid receptors, endocannabinoid ligands, and the enzymatic machinery that manages their biosynthesis, degradation, and transport, as well as all cells and neurological pathways that implicate endocannabinoid signaling (Fig. 1). It is involved in the control of the most important processes and creates homeostasis in the organism. This explains its ambiguous role in tumor development and tumor suppression. In the last decade, the ECS has been thrust into the forefront of anti-cancer research. As a result of the massive amount of data published on its dual roles in tumorigenesis as well as the suppression of tumor development and metastatic dissemination, it has emerged as a promising new target for the treatment of many different cancer subtypes. Although the search for cancer biomarkers typically favors single targets that allow for the exploit of a biochemical or genetic weakness, designating the vast ECS as a pharmacologically targetable entity has both benefits and drawbacks (Moreno et al. 2019).

The Discovery of Cannabinoid Receptors

The first ECS components discovered were Δ^9-tetrahydrocannabinol (THC) target areas in the brain. Cannabinoid receptors (CBR) were given the term for these receptors, but it was not until recently that it was discovered that they are among one of the most numerous neurotransmitter receptors in the entire organism. CB_1R

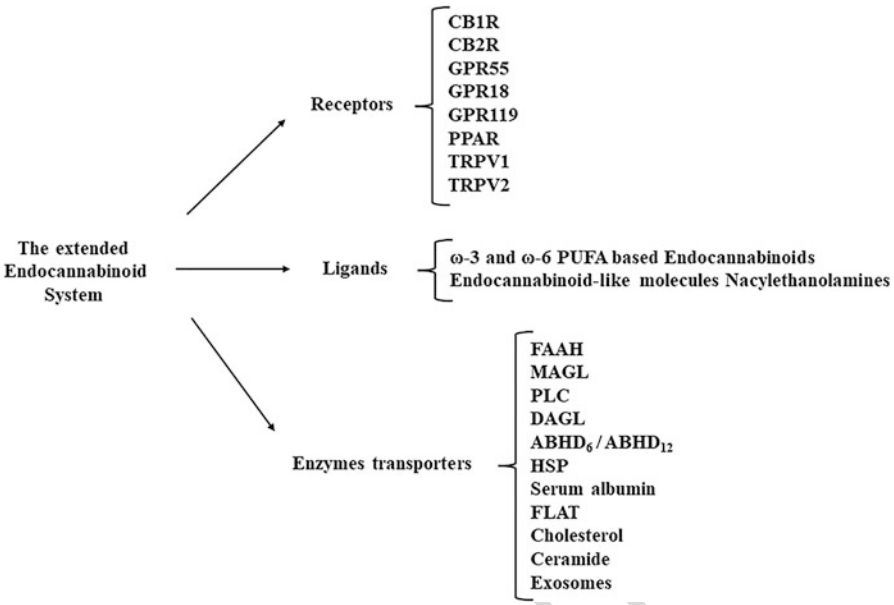


Fig. 1 Schematic representation of essential elements of the extended endocannabinoid system (ECS). *ABHD* alpha beta hydrolase domain proteins, *CBR* cannabinoid receptor, *DAGL* diacylglycerol lipase, *FAAH* fatty acid amide hydrolase, *FLAT* FAAH-like anandamide transporter, *GPR* G protein-coupled receptor, *HSP* heat shock proteins, *MAGL* monoacylglycerol lipase, *PLC* phospholipase C, *PPAR* peroxisome-proliferator-activated receptors, *PUFA* polyunsaturated fatty acid, *TRPV1* transient receptor potential cation channel subfamily V members 1, *TRPV2* transient receptor potential cation channel subfamily V members 2

was the first discovered and cloned receptor, and it was concluded that THC produces a presynaptic block of typical endogenous signaling of this receptor, resulting in the known psychotropic effects in the central nervous system (CNS). The second identified ECS receptor, designated CB_2R, was discovered in non-CNS localizations, predominantly on immune system cells. This discovery was a promising beginning for the investigation of the other elements involved in cannabinoids' non-psychotropic effects, as it was discovered that CB_2R ligands are responsible for their identified immunomodulatory effects (Acharya et al. 2017). Both receptors are members of a large family of class A rhodopsin-like G protein-coupled receptors (GPCR), which play a crucial role in signal transduction process between a variety of intracellular signaling molecules and external signals. They are excellent candidates for drug development since they are involved in several physiological and pathological processes. Both receptors are also expressed peripherally, where they regulate critical functions in the liver, lymph nodes, bones, skin, kidneys, reproductive system, lungs, gastrointestinum, and other organs. On chromosome 6 (6q15), the human central cannabinoid receptor gene 1 (CNR1) encodes three isoforms of the 60 kDa CB_1R protein. It is expressed in areas of the CNS that regulate memory and learning, emotions, sensory, motor behavior, and endocrine activities, as well as in

peripheral nerves and other non-neural sites (Atwood and MacKie 2010). The majority of CB1R are found presynaptically and are mostly coupled to inhibitory $G_{i/o}$ proteins, which, among other effects, inhibit adenylate cyclase and cause a reduction of intracellular cAMP levels. Endocannabinoids inhibit the release of excitatory and inhibitory neurotransmitters (GABA, glutamate, dopamine, acetylcholine noradrenaline, D-aspartate, etc.) by activating CB_1R at the membranes of glutaminergic and GABAergic neuron. As a result, CB_1R and its ligands contribute to neurotransmission plasticity and are desirable pharmacological targets. Multiple isoforms of a 40 kDa CB_2R protein are encoded by the human central cannabinoid receptor gene 2 (CNR2), which is found on chromosome 1 (1p36.11). Additionally, CB_2R are primarily found presynaptically and mainly coupled to inhibitory $G_{i/o}$ proteins, which means that 2-AG and other CB_2R ligands play a variety of roles in the control of peripheral neurotransmission and immune responses. The CB_2R was initially discovered in the immune system, but its expression was later discovered in other cell types. It is commonly known that CB_2R is present in microglial cells during neuroinflammation, but it has also been found, in astrocytes and some subpopulations of neurons (Atwood and MacKie 2010). The selective targeting of CB_2R in the CNS and the periphery may be a potential strategy for the treatment of many neurological disorders, because agonists that bind to CB_2R typically lack the psychotropic effects seen with CB_1R agonist-based therapy (Moreno et al. 2019). G protein-coupled receptors 18 (N-arachidonyl glycine receptor, GPR18), 55 (GPR55), and 119 (glucose-dependent insulinotropic receptor, GPR119) as well as the transient receptor potential cation channel subfamily V members 1 and 2 (TRPV1 and TRPV2) are additional receptors that respond to various cannabinoid ligands (Veilleux et al. 2019; Moreno et al. 2021).

The tumorigenic potential and signaling characteristics of the cell are altered by changes in CBR expression and activation, as well as by their capacity to form distinct functional heteromers with numerous other receptors, resulting in pharmacologically diverse responses to their stimulation. So, in various tumor subtypes, which are frequently pathologically driven by different biological causes, the same ECS component can have both protective and pathogenic effects (Moreno et al. 2020).

Cannabinoid Receptor Heteromers

The oligomerization concept is changing the classical views of GPCR physiology and pharmacology. Oligomerization implies that GPCRs can form macromolecular complexes constituted by the same (homomer) or different (heteromer) receptor protomers with functional properties clearly different from those of the individual components. The International Union of Basic and Clinical Pharmacology has established three consensus criteria to identify real GPCR heteromers (Kenakin et al. 2010). According to the first criterion, the heteromer elements must co-localize to the same subcellular compartment and physically interact in native tissues (Gomes et al. 2016). The second consensus criterion stipulates those specific properties of the heteromers, like trafficking, ligand binding, and signaling, which must be different from those associated with the individual protomers (biochemical

fingerprint). Some of these specific properties are positive and negative cross talk (co-stimulation with agonists of both receptors produce an additive or synergic effect or a substrative or anergic effect, respectively) and cross-antagonism (antagonist's binding to one of the receptors blocks signaling of the interacting receptor). Finally, criterion 3 proposes that disruption of the heteromer results in a loss of interaction and, thus, to a loss of the heteromer's distinctive biochemical fingerprint (Gomes et al. 2016). Even though many GPCR heteromers have been discovered using heterologous cell lines, only very few of them fit all three criterions. This is primarily due to the problems in studying these structures in native tissues due to the lack of selective and sensitive enough tools capable of detecting evidence of these endogenous heteromers in vivo and demonstrating that they are close enough to interact. As a result, for a GPCR heteromer to be accepted, at least two out of three criteria must be met.

Cannabinoid receptors have now been described as constituents of particular GPCR receptor heteromers, which is confirmed by various techniques (Casadó et al. 2010; Čavić et al. 2011). Details on currently known literature data on heteromers in various cell types have recently been extensively reviewed (Moreno et al. 2020). Previous research has shown that CB_1Rs can interact with other GPCRs, including orexin OX_1 receptors (generating positive cross talk and cross-antagonism by orexin), opioid μ and δ receptors (producing negative cross talk between receptors), dopamine D_2 (generating a change in the coupling from G_i to G_s), adenosine A_{2A} receptors, D_2 and A_{2A} receptors at the same time (producing negative modulation of the function of D_2 receptor by CB_1 and A_{2A} agonists), angiotensin AT_1 receptors (with increased AT_1 receptor signaling) and CB_2R (the coactivation of both receptors results in negative cross talk and bidirectional cross-antagonism in neuronal cells in vitro and in vivo), adrenergic β_2 receptor (adrenergic agonists inducing CB_1R internalization), and 5-HT_{2A} serotonin receptor (Moreno et al. 2018, 2019, 2020). CB_1R can also form heteromers with the cannabinoid-related orphan receptor GPR55 in cells. In contrast to CB_1R, little is known about the existence and functional significance of CB_2R heteromers; nevertheless, there is proof of its interaction with GPR55 in transfected cells (Moreno et al. 2014) and breast cancer cells with specific pharmacological and signaling properties (its expression alters cannabinoid signaling in such a way that, when directly targeted with the right doses of THC, tumor development may be inhibited, both in vitro and in vivo) (Moreno et al. 2014). In the context of metastatic disease, CB_2R modulates tumor cell migration and invasion via interacting with the chemokine receptor CXCR4 in human prostate and breast cancer cells with new pharmacologic properties (Scarlett et al. 2018). Additionally, HER2-CB_2R heteromers are a novel therapeutic target for HER2+ breast cancer because they interact with the tyrosine kinase receptor (TKR) human V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 2 (HER2) in human breast. Therefore, the CXCR4-CB_2R, GPR55-CB_2R, and HER2-CB_2R protein complexes possess specific pharmacological and signaling properties, and their modulation could have an impact on the ECS's ability to inhibit tumor growth (Moreno et al. 2019).

The Discovery of Endocannabinoids

Numerous cells in the human organism express endocannabinoids and endocannabinoid-like molecules (ECLs), and their levels reflect the metabolic adjustments required for the maintenance of homeostasis as well as the body's reaction to pathological stimuli. These molecule groups are highly diverse, in terms of their structure, the type of receptor they can excite, and the non-canonical biochemical processes they can influence. Anandamide (N-arachidonoyl ethanolamide, AEA) and 2-arachydonoyl glycerol (2-AG), which are both derived from ω-6 arachidonic acid (ARA), an essential polyunsaturated fatty acid (PUFA), were the earliest and most prevalent conventional endocannabinoids. In addition to AEA and 2-AG, other unsaturated lipid-based molecules have been categorized as endocannabinoids because of their function and comparable high levels, the ECLs N-acylethanolamines (NAES), like N-palmitoyl-, N-oleoyl, and N-linoleoyl-ethanolamine (PEA, OEA, and LEA); 2-acyl-glycerols (2-AcGs) like 2-oleoyl and 2-linoleoyl-glycerol (2-OG and 2-LG); prostaglandin ethanolamides; prostaglandin glycerol esters; and omega-3 endocannabinoids that originated from docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (like docosahexaenoyl ethanolamide (DHA-EA), docosahexaenoyl-glycerol (DHG), eicosapentaenoyl ethanolamide (EPA-EA), and eicosapentaenoyl-glycerol (EPG)) (Watson et al. 2019). The importance of these tiny molecules is demonstrated by the existence of their signaling pathways in even the most basic organisms, where they control a variety of essential functions. They are created and activated by the body when necessary, and they regulate adaptive cellular responses to a variety of endogenous and exogenous stimuli that threaten internal homeostasis (Moreno et al. 2019, 2021). The endocannabinoidome, also known as the enlarged ECS, constitutes a vast network made up of the endocannabinoids and ECLs as well as the related metabolic enzymes and molecular targets (Veilleux et al. 2019). The metabolic complexity of such a vast network of molecules in each environment must be carefully assessed in each context, which may be aided by the increasing capacity of "omics" approaches and contemporary sequencing technologies. Since endocannabinoid signaling effects are primarily limited to the local sites of its biosynthesis and release, the endocannabinoid signaling is not a typical example of neurotransmission. Their biosynthesis occurs before the stimuli, and they are kept in synaptic vesicles until required. They are carried back to the presynaptic neuron, after exerting their effect on the receptors of postsynaptic neurons, ending the brief reaction to a stimulus (Lu and MacKie 2016).

Because exogenous cannabinoids are frequently supplied in excess, they can assume the endocannabinoid transmission for longer periods of time and cause a variety of physiological consequences. Endocannabinoids have also been shown to have non-receptor regulatory effects, which add to the ECS's plasticity (Lu and MacKie 2016). Because CBRs' binding pocket is adaptable and can interact with ligands that do not precisely match the size and shape of the endocannabinoids they are designed to bind to, exogenous cannabinoids (both synthetic and plant-derived) bind to CBRs. Consequently, exogenous cannabinoids (allosteric, bitopic/dualsteric,

or bivalent ligands) can interfere with any of the endocannabinoid-regulated processes, and their effects have been thoroughly studied in a variety of contexts (including pregnancy/infertility, motor functions, CNS development, etc.). Some commonly used exogenous cannabinoids are THC and cannabidiol (CBD) (Moreno et al. 2019; Suryavanshi et al. 2021).

The Discovery of the Endocannabinoid Enzymatic and Transport Systems

The enzymatic machinery responsible for the biosynthesis, degradation, and transport of endocannabinoids was the final element of the ECS that was characterized in more detail. Endocannabinoids are lipid neuromodulators that are produced on-demand, allowing the ECS to act quickly and adaptively. The biosynthesis and degradation pathways of anandamide and 2-AG are totally different, demonstrating their distinct physiological functions. They are both derivatives of arachidonic acid, an essential PUFA, but whereas anandamide is primarily produced from N-arachidonoyl phosphatidyl ethanol through a variety of pathways, 2-AG is produced mostly from phosphatidyl inositol bis-phosphate via a calcium-dependent pathway affecting phospholipase C (PLC) and diacylglycerol lipase (DAGL) (Lu and MacKie 2016). Additionally, in the endoplasmic reticulum, fatty acid amide hydrolase (FAAH) primarily hydrolyses AEA, and monoacylglycerol lipase (MAGL) is the main hydrolytic degrader of 2-AG, with FAAH and also some serine hydrolases (ABHD6 and ABHD12) being proposed as secondary degraders (Savinainen et al. 2012). As endogenous cannabinoids are hydrophobic and cannot easily diffuse across the cytosol and cell membrane, their levels are also influenced by the efficiency of their uptake and effective transport within the cell. Depending on the type of cell, numerous transporters and molecules have been implicated in this process, including ceramides, cholesterol, serum albumin, heat shock proteins, and other fatty acid-binding proteins as FAAH-like anandamide transporter (FLAT). AEA is recycled back to the cytoplasm and is subjected to enzymatic hydrolysis once it has reached the target receptors on cells and begun to exercise its effects. CBD can interfere with AEA FAAH-mediated degradation, increasing available AEA levels and thus indirectly inducing a variety of non-psychoactive consequences (Moreno et al. 2019).

The Significance of the ECS in Physiological and Pathological Processes

The ECS is crucial for the maintenance and regulatory processes of several putative biochemical pathways. Regulation of homeostasis by fine-tuning of essential metabolic axes is characteristics for the ECS. As it is involved in many physiological and pathological processes, it has emerged as a useful pharmacological target, but its potential is still not fully explored. ECS ligands are useful for pain alleviation, which

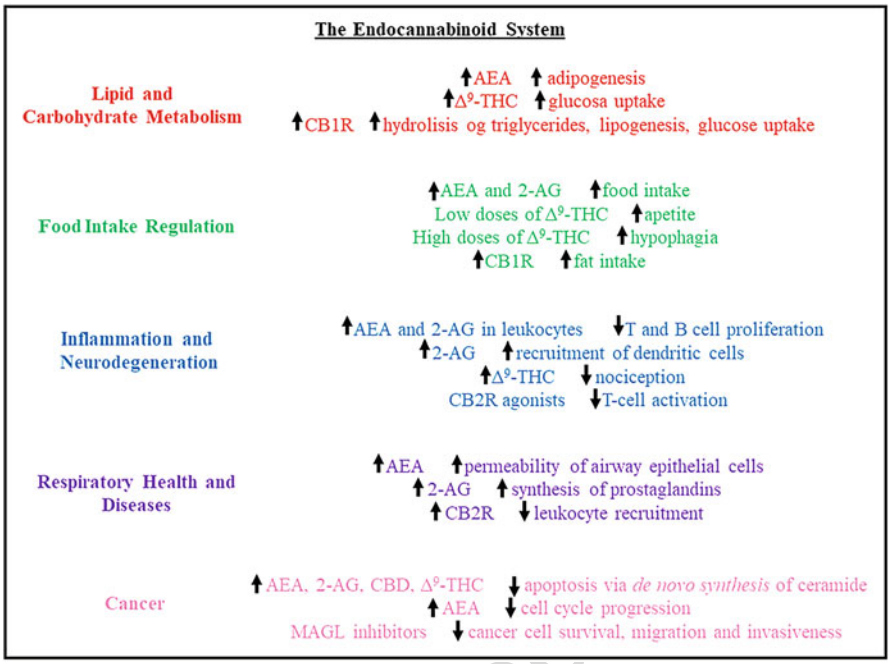


Fig. 2 A schematic representation of various roles of the endocannabinoid system in selected physiological and pathological pathways and processes

enables faster drug repurposing in other settings. Exogenous ECS ligands with psychotropic side effects are only a minor part of known potential ECS drugs, and this effect should be regarded as an obstacle in new studies (Martínez et al. 2020). In addition, various studies are exploring ECS-related drugs that are not able to cross the blood-brain barrier, thus controlling the possible side effects. Allosteric modulators and lower doses of known drugs are also being explored. The enzymatic machinery used by other biologically potent ligands might also lead to inaccurate interpretation of results of metabolic regulation and homeostasis and needs to be taken into account while exploring ECS ligands' broader application (Fig. 2) (Stasiulewicz et al. 2020; Moreno et al. 2021).

Lipid and Carbohydrate Metabolism

A positive correlation of soluble ECS components with metabolic disorder/obesity biomarkers was also reported (Simon and Cota 2017), contributing to the idea that ECS might be involved in the regulation of metabolic homeostasis (Moreno et al. 2021). As the ECS is localized both centrally and at the peripheral level, it is important for integrating commands coming from various brain regions, regulating the brain-periphery communication, and adjusting components involved in the

metabolism of glucose and lipids. When activated, it contributes to energy intake and storage, but once high-caloric food sources are present, anabolic activation of ECS uprise may lead to obesity and related complications. The appearance of hypertension, hypertriglyceridemia, and sometimes even insulin resistance ultimately might bring on metabolic syndrome and/or type 2 diabetes (Simon and Cota 2017).

Food Intake Regulation

Eating disorders and behavior related to ECS have been recently explored in several human studies. As ECS ligands are present in detectable amounts in blood, their assessment might be performed using a minimally invasive strategy. It is known that ECS is important for developing a preference to certain food types, as it modulates responses to taste and olfactory sensations, thus producing metabolic changes related to food consumption. Vice versa, the specific diet type in turn affects the levels of ECS components in the circulation and hence their mode of action and strength of effect. When fat is present in the oral cavity, it leads to the production of jejunal ECS components, increasing fat appetence. Reports of gastric CB_1R activation have been linked to ghrelin secretion, inducing the perception of fat taste which augments fat intake (Simon and Cota 2017). Close interactions were also detected between ECS ligands and hormones involved in the regulation of energy balance, as glucocorticoids, ghrelin, and leptin. WAT ECS ligand levels were found to be negatively affected by insulin and leptin, an effect that might be missing during insulin or leptin resistance, when ECS overactivity and fat access can be observed. Studies have also pointed to a strong association between the ECS and the gut microbiome, when exploring sets of genes, proteins, and metabolites derived from the action of intestinal microflora. Moderate and prominent lifestyle modifications might provide an explanation for the development of metabolic syndrome through activation of cross-talk mechanisms between the gut microbiome and ECS components (Di Marzo and Silvestri 2019; Moreno et al. 2021).

Inflammation

While various attempts to elucidate the role of ECS in inflammasome modulation leading to chronic inflammatory diseases have been made, no results have yet been confirmed in prospective studies. Although it is known that cannabinoids (especially THC and CBD and synthetic cannabinoids) have anti-inflammatory properties, exact mechanisms of these interactions remain to be explored in detail (Jakowiecki et al. 2021). Changes in ECS component levels and their modifications have been detected in a number of chronic inflammatory diseases and associated states as cancer, autoimmune diseases, diabetes mellitus, etc. (Leuti et al. 2020). Endocannabinoids are generally considered as potent immunosuppressors in chronic conditions, through activation of ECS-related signaling, regulation of inflammatory cytokine levels, and chemotaxis and proliferation of immune cell populations, among others (Chiurchiu et al. 2015).

ECS is also considered as one the most important immunomodulators in the brain. The observed neuroprotective effects of ECS-based drugs are achieved through inducing a decrease of the level of neuroinflammation. It leads to a stabilization of main processes that deregulate neuronal homeostasis, as oxidative stress and apoptosis. As CB_2R is found both on microglia and brain-infiltrating cells of the immune system, most immunoregulatory roles have been attributed to its activation. However, there have been reports on the involvement of CB1R, while modelling situations as traumatic brain injury, Alzheimer's disease (AD), and multiple sclerosis models (Leuti et al. 2020). Increased brain levels of AEA and 2-AG achieved through the inhibition of their main catabolic enzymes as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) might provide an efficient approach to regulate the immune response in some conditions (Chiurchiù et al. 2018; Leuti et al. 2020; Moreno et al. 2021). CBD has also been reported as a strong antioxidant and neuroprotectant in some neurological diseases as AD and Parkinson's disease (PD).

Respiratory Health and Diseases

Respiratory epithelial cells distributed along airway paths constitute the basis of respiratory health acting both as a physical and biological border that pathogens need to pass. The lack of proper function of ECS components in these cells is considered as a prominent metabolic issue contributing to destabilization of healthy lung function and physiological processes activated upon the presence of malicious stimuli (Fantauzzi et al. 2020). It has been reported that AEA can lead to a higher permeability of the airway epithelium though the formation of arachidonic metabolites with biological activity within the cells, avoiding the classical canonical axis (Shang et al. 2016). 2-AG might also yield prostaglandins in the airway epithelium which neutrophils and eosinophils can convert into leukotrienes B4 and C4, thus establishing an interconnection between ECS and prostaglandin pathways (Turcotte et al. 2016). CB_2R has been reported to interact with viral pathogens in the lungs leading to overactivation of the respiratory ECS enhancing the response to respiratory infections. Introducing exogenous cannabinoids through inhalation pathways might lead to respiratory metabolic symptoms characteristic for asthma and pulmonary fibrosis, among others. As the interplay of ECS and other regulatory mechanisms in these diseases is rather complex and not fully understood, a disbalance of joint metabolites that bind to receptors of respiratory system cells can ultimately induce a range of symptoms depending on the levels of activated physiological responses on one side and inflammation on the other (Moreno et al. 2021).

Cancer

The ECS has primarily been linked with palliative application in the oncological setting, although its components and the corresponding biochemical network are considered as prognostic and predictive factors of various tumor-related states and

anti-cancer treatments. It comes as no surprise that the ECS network is considered as a prominent pharmacological target, with a growing potential that has not yet been fully explored. The ECS is involved in the regulation of putative biochemical pathways important for breast, gastrointestinal, and CNS tumors among others, as well as in proliferation and apoptosis, immune response, and angiogenesis (Moreno et al. 2020). Several studies using in vitro and in vivo models, as well as clinical studies, have explored its potential in anti-cancer approaches. However, the exact effect it induces and clinical utility depend greatly on the tumor subtype and interactions with metabolic pathways highlighted in that specific setting. A complex, multilateral metabolic exchange occurs in a pro-tumorigenic cell, rendering the ECS's involvement in the development and progression of a cancerous state in need of careful evaluation in each cancer cell type in question (Moreno et al. 2021).

The Role of ECS in Cancer Development

ECS regulatory mechanisms are in connection with almost all prominent cancer pathways. As various ECS components can act as regulators of proliferation, apoptosis, angiogenesis, and inflammation depending on cancer subtype, the changes in their levels in tumors are in close connection to aggressiveness of the tumor. Overexpression and overactivation of ECS components can be a tumorigenic factor, but the regulatory system is as complex that the overall effects depend on a variety of factors. It has been reported that cannabinoid receptors, some cannabinoid ligands, and ECS enzymatic components have altered expression levels in tumors which contribute to their aggressiveness depending on the tumor type (Moreno et al. 2020).

Cannabinoid Receptors in Cancer

The expression of most frequently analyzed cannabinoid receptors CB_1R and CB_2R in tumor and normal samples analyzed using UALCAN, a comprehensive, interactive web resource for the analysis publicly available in the Cancer Genome Atlas (TCGA) data, is presented in Fig. 3 (Chandrashekar et al. 2017; UALCAN 2021).

As presented in Fig. 3, cannabinoid receptors 1 and 2 are expressed on the plasma membrane of a wide range of normal and cancer cells. Detailed in silico data mining analyses presented in Table 1 confirmed that cannabinoid receptors are significantly enriched in some cancers, while they are downregulated in others, which contributes to the hypothesis that their protective/cancer-promoting role depends on the tumor subtype in question.

Once CBRs are activated, it can induce a significant downregulation of cancer growth which suggests that ECS might have prominent tumor-suppressive characteristics. These data are strengthened by in vivo studies reporting that the absence of CB_1R on cells contributes to cancer growth and that higher ECS ligand levels reduce the size and spread of precancerous conditions (Velasco et al. 2015). Activation of

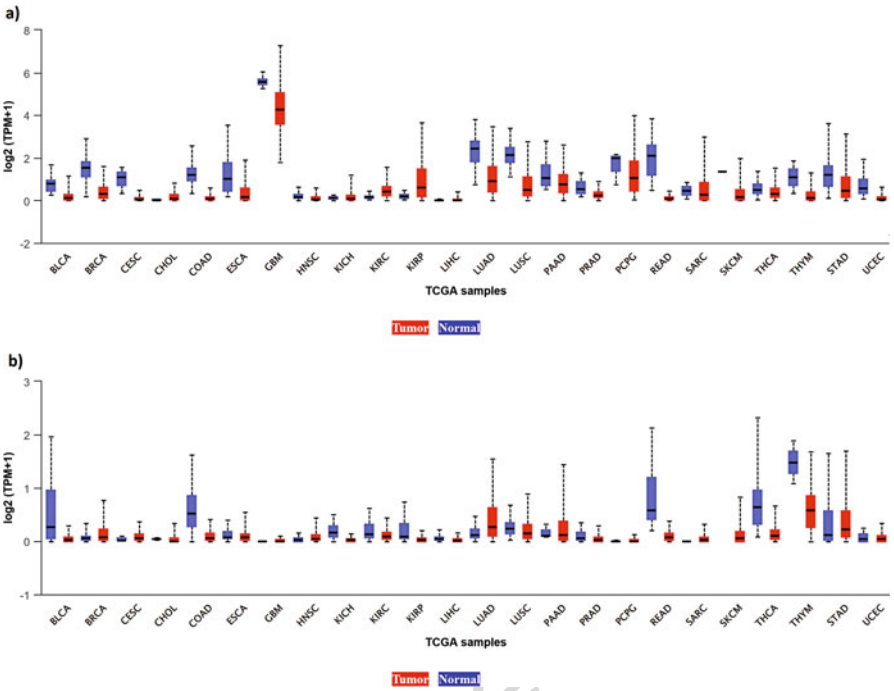


Fig. 3 The expression of CB_1R (**a**) and CB_2R (**b**) across TCGA cancers (tumor and normal samples) according to the UALCAN database. *BLCA* bladder urothelial carcinoma, *BRCA* breast invasive carcinoma, *CESC* cervical squamous cell carcinoma, *CHOL* cholangiocarcinoma, *COAD* colon adenocarcinoma, *ESCA* esophageal carcinoma, *GBM* glioblastoma multiforme, *HNSC* head and neck squamous cell carcinoma, *KICH* kidney chromophobe, *KIRC* kidney renal clear cell carcinoma, *KIRP* kidney renal papillary cell carcinoma, *LIHC* liver hepatocellular carcinoma, *LUAD* lung adenocarcinoma, *LUSC* lung squamous cell carcinoma, *PAAD* pancreatic adenocarcinoma, *PRAD* prostate adenocarcinoma, *PCPG* pheochromocytoma and paraganglioma, *READ* rectum adenocarcinoma, *SARC* sarcoma, *SKCM* skin cutaneous melanoma, *THCA* thyroid carcinoma, *THYM* thymoma, *STAD* stomach adenocarcinoma, *UCEC* uterine corpus endometrial carcinoma

CBRs might initiate apoptosis of cancer cells by regulating RAS/MAPK and PI3K-AKT axes, as well as TNFa-dependent ceramide synthesis, COX-2-related cell death, and other reported scenarios (Moreno et al. 2019).

CBR heteromers are attractive pharmacological targets as they might contribute to the pathophysiology of cancer-related disorders but also be used as prognostic and predictive biomarkers (Laezza et al. 2020; Moreno et al. 2020). The modulation of heteromers like CXCR4-CB_2R, GPR55-CB_2R, and HER2-CB_2R has been reported to exert anti-cancer activity in breast and prostate cancer, specifically. For example, once CXCL12 ligand binds to its receptor CXCR4, a CXCR4-induced cell migration followed by adhesion occurs. When both CXCR4 and CB_2R agonists are applied concomitantly, this leads to the inhibition of the observed CXCR4 agonist effect, as the signaling molecule taking over the cascade is the functional CB_2R-CXCR4

t.1 **Table 1** Expression of cannabinoid receptors CB_1R and CB_2R in cancer subtypes comparing normal vs primary tissue according to the UALCAN database

Receptor	Upregulated*	*p* value	Downregulated*	*p* value
t.2 CB_1R	Cholangiocarcinoma	0.0316	Breast invasive carcinoma	<0.0001
t.3	Kidney chromophobe	0.0163	Colon adenocarcinoma	<0.0001
t.4	Kidney renal clear cell carcinoma	<0.0001	Lung adenocarcinoma	<0.0001
t.5	Kidney renal papillary cell carcinoma	<0.0001	Lung squamous cell carcinoma	<0.0001
t.6	Liver hepatocellular carcinoma	<0.0001	Prostate adenocarcinoma	<0.0001
t.7			Rectum adenocarcinoma	0.0098
t.8			Stomach adenocarcinoma	0.0372
t.9			Thyroid carcinoma	0.0305
t.10			Uterine corpus endometrial carcinoma	0.0154
t.11 CB_2R	Breast invasive carcinoma	0.01554	Colon adenocarcinoma	0.0007
t.12	Head and neck squamous cell carcinoma	<0.0001	Kidney chromophobe	0.0437
t.13			Kidney renal papillary cell carcinoma	0.0218
t.14			Rectum adenocarcinoma	0.0283
t.15			Thyroid carcinoma	<0.0001

t.16 *Only data significant at $p < 0.05$ are presented

heteromers. This ultimately leads to inhibition of prostate cancer cell migration and adhesion. Dose-dependent effects have also been reported. When THC acts through the CB_2R-GPR55 heteromer in low levels, it induces a CB_2R signaling enhancement. At higher levels, THC targets the GPR55 and exerts an antagonist role through cross-antagonism, effectively inhibiting CB_2R which leads to a downregulation of tumor growth, in vitro and in vivo. THC can also act through the HER2-CB_2R heteromer inactivating HER2 and leading to a prominent anti-cancer effect. All these reports increase the burden of scientific evidence that ECS heteromers might be the real targets in future clinical studies in the oncological setting.

Cannabinoid Ligands in Cancer

The use of ECS components as anti-cancer drugs depends on the effect they produce in a specific cancer cell, as both protective and damaging outcomes to cellular homeostasis might occur. Cannabinoid ligands have the ability to act as regulatory molecules in lipid metabolism unrelated to cancer, leading to a conditionally accidental contribution to cancer development and progression (Zhang et al. 2016). It has been reported that ECS ligands interact with chemo- and targeted therapeutics, which needs to be taken into consideration for concurrent drug application.

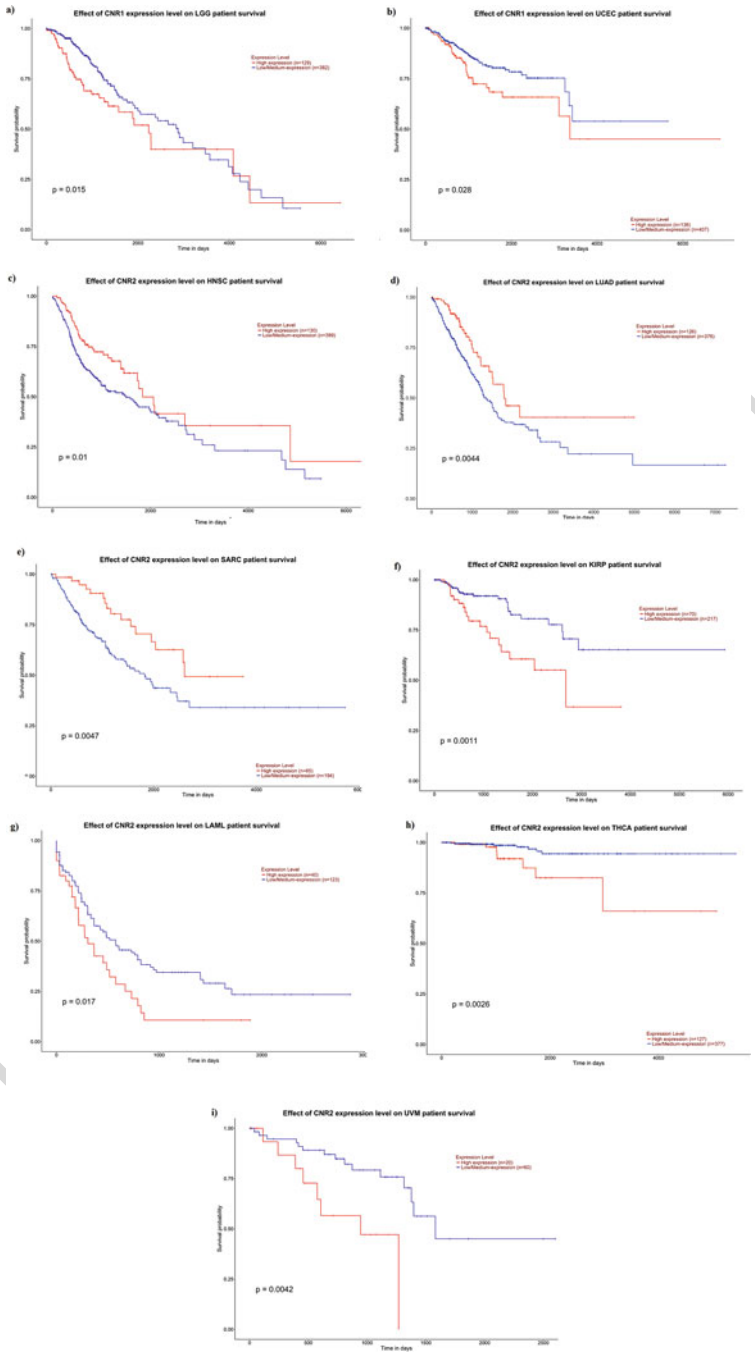
Cell line and animal experimental models of cancer have shown that cannabinoids exert anti-cancer effects. Synthetic cannabinoid ligands show a levelled affinity for CBRs as natural ECS ligands and even higher selective affinities one receptor or the other, as methanandamide for CB_1R or JWH-133 for CB_2R. All these data brought on a load of preclinical evidence that one stimulated CBRs might exert a general anti-cancer effect. Initiation of apoptosis, inhibition of uncontrolled cell growth is the usual mode of cannabinoid effect, in a variety of cancer cell types tested in in vitro settings. In vivo studies further confirmed these observations and additionally shed light on the role of ECS in inhibition of angiogenesis and metastasis. Reports of tumor-promoting properties of ECS components in vitro have been linked with interactions with the immune system leading to its inhibition as a tumor-suppressor agent (McKallip et al. 2005).

Cannabinoid Enzymes and Transporters in Cancer

ECS enzymes and transporter molecules have been evaluated as therapeutics, but the changes they can induce on ECS component metabolism and transport might be unpredictable. Thus, limited success of these studies has been reported. Alterations of ECS metabolic networks through changes in enzyme/transporter activity and expression affect the levels of ECS ligands. This might exert a general protective outcome in scenarios of deregulated enzyme levels as is the case of tumorigenesis. This effect has been reported to MAGL in colorectal cancer (Pagano et al. 2017). The significance of this approach is highlighted by the fact that various inhibitors of FAAH, MAGL, and ECS transporters are in testing phases of experimental and clinical studies, both as single agents and in combination (Chicca et al. 2017; Granchi et al. 2017). The development of more pharmacological options of this type needs to include a strict evaluation of the CNS and systemic adverse effects to avoid learning and memory alterations upon treatment (Panlilio et al. 2016; Moreno et al. 2019).

The Role of ECS in Cancer Progression and Prognosis

The effect of cannabinoid receptors on the prognosis of cancer patients was evaluated by analysis of TCGA data on patient survival (Fig. 4). Upregulated CNR1 expression coincided with shorter patient survival in brain lower-grade glioma ($p = 0.015$) and uterine corpus endometrial carcinoma ($p = 0.028$). Upregulated CNR2 expression coincided with longer patient survival in head and neck squamous cell carcinoma ($p = 0.010$), lung adenocarcinoma ($p = 0.004$), and sarcoma ($p = 0.005$) and with shorter survival in kidney renal papillary cell carcinoma ($p = 0.001$), acute myeloid leukemia ($p = 0.017$), thyroid carcinoma ($p = 0.003$), and uveal melanoma ($p = 0.004$). Thus, the survival of patients with different cancer subtypes does not seem to be influenced directly by the expression of cannabinoid receptors, but rather



AU7 **Fig. 4** Survival curves of cancer patients according to TCGA data on expression of CNR1 and CNR2 available through the UALCAN database (Chandrashekar et al. 2017; UALCAN 2021). (**a**) LGG, brain lower-grade glioma; (**b**) UCEC, uterine corpus endometrial carcinoma; (**c**) HNSC,

by a complex interaction of their expression levels and activation/regulation via ligands and possibly heteromerization.

Mechanisms of action of ECS components that affect cancer progression include the regulation of the cell cycle, apoptosis, autophagy, epithelial to mesenchymal transition, adhesion, invasion, metastasis, and angiogenesis (Pagano et al. 2021).

The Role of CBRs in Cancer Progression and Prognosis

Reports documenting positive effects of downregulation of CBRs exist, as described for genetic ablation of CB_1R and CB_2R to lower the effect of UV light in the initiation of skin cancer or loss of expression of CBRs which might induce faster tumor growth. However, more literature data supports the view that various ECS components have anti-cancer properties, as over-expression or activation of CBRs and higher cannabinoid levels have been reported to slow down tumor growth. All these effects have been reviewed in detail in Moreno et al. presenting supporting literature data (Moreno et al. 2019).

From the described heteromers, the GPR55-CB_2R overexpression and heteromerization have been reported in in vitro studies using human breast cancer cells and others. By applying the cannabinoid ligand THC, downsizing of tumor mass has been reported in vitro and in vivo. The $GXCR_4$-CB_2R heteromer has also been reported as significant in human breast and prostate cancer cells (Scarlett et al. 2018). The CXCR4 receptor is known to enhance cellular mechanism important for proliferation and migration, and its activation is prominent in cells exhibiting the potential for local and distant metastatic spread. However, if agonist of both CXCR4 and CB_2R are applied together in vitro, a prominent downregulation of ERK1/2-dependent cellular migration induced by CXCR4 is reported, highlighting the functional cross talk between the two receptors in the heteromer (Coke et al. 2016). Thus, an inhibition of the effect of CXCR4 ligands is observed by applying different cannabinoids that activate CB_2R and might be used to regulate the metastatic potential of cancer cells. Prominent pathways associated with this effect in prostate cancer cells employ the $G\alpha_{13}$/RhoA and integrin α5 signaling axes, which in turn regulate the cytoskeletal components involved in cell migratory properties and adherence to the extracellular surrounding environment, respectively. In breast cancer, the existence of the HER2-CB_2R heteromers has been documented. Up to 30% of primary breast cancer cells have either overexpressed HER2 or exhibit its amplification at the genetic level, which predicts shorter survival and poor response to anti-cancer therapy. CB_2R is also overexpressed in breast cancer and connected to more aggressive cancer forms, sometime through interaction with HER2. In the

Fig. 4 (continued) head and neck squamous cell carcinoma; (**d**) LUAD, lung adenocarcinoma; (**e**) SARC, sarcoma; (**f**) KIRP, kidney renal papillary cell carcinoma; (**g**) LAML, acute myeloid leukemia; (**h**) THCA, thyroid carcinoma; (**i**) UVM, uveal melanoma

absence of ligands, CB_2R assumes the role of the main receptor in the heteromer, by regulating HER2 signaling (Pérez-Gómez et al. 2015). In the presence of CB_2R ligand THC, the HER2-CB_2R complex is disrupted by binding to CB_2R with high selectivity, which induces the inactivation of HER2 (by disrupting HER2-HER2 dimers) and its degradation, ultimately activating anti-cancer signaling pathways. Strategies involving double stimulation of both receptors concurrently might induce synergistic anti-cancer effects, which will be further explored in section "The Role of ECS in Response to Standard Anti-Cancer Treatment".

Thus, the protein complexes that are formed between various CBRs and other membrane receptors contribute to the value of ECS components as prognostic markers, as well as promising new, and still under explored, targets for anti-cancer treatment.

The Role of Cannabinoid Ligands in Cancer Progression and Prognosis

Concerning cannabinoid ligands, in vitro and in vivo evidence supports their involvement in the regulation of signaling pathways essential for cell survival, growth, and spread, as well as proliferation, angiogenesis, and cell cycle (Nigro et al. 2021). As endocannabinoids are crucial for the regulation of lipid metabolism AU8 and other prominent signaling pathways important in cancer, altered levels in some subtypes and disease stages have been reported (Zhang et al. 2016). The extent of their contribution to disease progression and metastatic spread is not easily determined but has been explored in various studies starting from the 1980s/1990s. The general mode of action is cancer cell death by apoptosis, as well as inhibition of cellular proliferation, thus contributing to an overall beneficial effect against cancer progression and a more favorable prognosis. However, there are studies that have documented that cannabinoid ligands might exert tumor-promoting activity in vitro, both directly acting through CBRs and indirectly negatively affecting the immunological surrounding that should contribute to an overall tumor-suppressor state (Braile et al. 2021). The literature data is still conflicting, as there have been reports that cannabinoids have the ability to enhance the tumor surveillance in specific cancer subtypes in vitro (Simmerman et al. 2019).

When the induction of autophagy is concerned, it usually involves the action of p8. However, upregulation of AMPK and downregulation of the AKT-mTORC1 axis induced by TRIB3 have also been reported in some cancers as hepatocellular, and downregulation of the AKT signaling in melanoma and breast cancer (Moreno et al. 2020). The activation of CBRs in melanoma, gliomas, and thyroid carcinomas has been negatively linked with VEGF-induced angiogenesis and positively correlated with the inhibition of cancer cell adhesion and invasion in breast, lung, and cervical cancer (Pagano et al. 2021). The endocannabinoid anandamide has been shown to bind to CB_1R and block the cell cycle progression, inhibiting the activity of adenylyl cyclase which enhances the Raf-1/ERK/MAPK signaling axis without inducing cancer cell death.

The Role of Other ECS Components in Cancer Progression and Prognosis

The enzymatic machinery involved in various ECS-related pathways has also been reported to affect tumor development and growth. As ECS enzymes affect the endocannabinoid biosynthesis, catalytic, and transport processes, the range of outcomes they can exert depends in great level on the cancer type in question and the surrounding microenvironment (Vuletić et al. 2021). Increased availability of cannabinoid ligands by changes in the activity of ECS enzymes might lead to protective effects, which is even more pronounced in cases where ECS components have been deregulated by cancerogenesis. For colorectal cancer, overexpression of MAGL has been reported which was correlated with poor patient prognosis (Pagano et al. 2017). In brain tumors as glioma and endometrial cancer, FAAH and MAGL are downregulated which affect the production of endocannabinoids as AEA and 2-AG. In prostate cancer, high expression of FAAH was noted. A similar situation was reported for pancreatic ductal adenocarcinoma, where high levels of FAAH and MAGL correlated with prolonged patient survival (Pagano et al. 2021).

ECS as a Novel Target in Cancer: Status and Future Perspectives

The vast biochemical milieu of effects the ECS components can exert and regulate contributes to its role in pivotal processes important both for cancer progression and thus prognosis of cancer patients. Besides the reported direct and indirect effects on proliferation, apoptosis, and angiogenesis and metastatic potential of cancer cells, their involvement in the regulation of inflammatory pathway might be crucial depending on the specific tumor subtype in question (Braile et al. 2021). Thus, their significance as novel targets in cancer, as monomers and heteromers, must be evaluated in detail depending on the cancer subtype, taking into account biased agonism which might change the overall effect of potential treatment (Laprairie et al. 2017).

Some tumor cells are rich cannabinoid receptors and are attractive anti-cancer targets, although their level of expression is not always in direct correlation with tumorigenicity (Soderstrom et al. 2017). Various biochemical pathways might be affected while targeting CBRs producing several outputs important for controlling the growth and spread of cancer cells. They include but are not limited to the deregulation of endoplasmic reticulum stress-inducing factors, TNFα-linked synthesis of the sphingolipid ceramide, cell death dependent on regulation of COX-2, inhibition of angiogenesis, deregulation of RAS/MAPK and PI3K-AKT axes (Pagano and Borrelli 2017), etc. However, the transfer of information when targeting CBRs needs to be rigorously inspected, as they foster a number of allosteric connections in the plasma membrane and tend to form heteromers specific for the cancer subtype in question (Moreno et al. 2020). It is currently widely accepted that in most biochemical scenarios, the effective true cannabinoid receptor targets are not monomeric structures, but heteromeric complexes comprised of two or more same and different receptor monomers in the plasma membrane (Sánchez-Soto et al. 2018). The biochemical

plasticity that the heteromers induce is even more pronounced when considering the existence of allosteric cannabinoid ligands binding to conserved orthosteric sites present on different GPCRs or by the existence of biased cannabinoid ligands. Strategies targeting two receptors in a heteromer have also been explored. When considering the mentioned HER2-CB_2R heteromer, it was reported that inactivation and disruption of HER2 can be initiated by THC binding to the CB_2R component of the heteromer (Blasco-Benito et al. 2019). Thus, a control of the oncogenic properties of one receptor, as HER2 in breast cancer, might be achieved through targeting the corresponding monomer pair in the heteromer entity, unraveling new and specific anti-cancer mechanism. Cannabinoids can also act through non-receptor-based mechanisms and induce significant anti-cancer effects contributing to control of cancer invasiveness and metastatic spread. For CBD it has been shown to activate autophagy through ERK1/2 activation and AKT downregulation, using the autophagy initiator ULK1 and without the involvement of mTORC1, pointing to a non-canonical pathway of action in neuroblastoma (Vrechi et al. 2021).

Using cannabinoids as oncological therapeutics is a promising area of research. They have been shown to exhibit protective effects against cancer cell growth and spread, contributing to an overall state of homeostasis. Most available literature data from clinical trials using cannabinoid ligands presented in reviews explored the roles of cannabis, cannabinoids, marijuana, THC, CBD, dronabinol, marinol, nabilone, and nabiximols. The reviews describe data on the main cannabinoid applications in alleviating neuropathic or chronic pain, as well as it well documented uses in controlling nausea, vomiting, appetite, intestinal dysfunction, glaucoma, and similar conditions. Cancer studies are also covered, although none of the clinical trials have yet to lead to a globally accepted use of any ECS component in anti-cancer treatment. Most clinical studies were conducted on terminal patients who consented to trials after exhausting all other possible treatments, and the main use of cannabinoids was in control of cancer-related pain. However, these studies showed that cannabinoids can also participate in the control of tumor growth, acting both alone and in combination with other cytotoxic drugs (Mangal et al. 2021). The potential of many plants and endogenous and synthetic cannabinoids have been explored using in vitro and in vivo cancer models. Although glioma cells have been most used as a model for evaluating the anti-cancer pathways that cannabinoids modulate, many other cancer subtypes have been used as well, such as lung cancer, thyroid epithelioma, lymphoma, and melanoma. Literature data supports the anti-cancer potential of cannabinoids, through the induction of apoptosis, activation of CBR-dependent de novo synthesis of pro-apoptotic sphingolipid ceramide, autophagy (Lee et al. 2021), regulation of cancer angiogenesis (Ladin et al. 2016), inhibition of cell proliferation and epithelial-mesenchymal transition (Milian et al. 2020), and interaction with the tumor immune microenvironment (Braile et al. 2021). The outcome of applying cannabinoids varies on the type of ligand, its concentration, affinity for the receptors expressed in that specific cancer subtype, the presence of heteromers, tumor stage, and the general immune state of the tumor surroundings.

Using ECS enzymes and transporters as targets has also been explored as anti-cancer strategy, but with limited success. As they might have a vast range of CNS-related and systemic adverse effects, their use has so far been experimental. Many modulators of

FAAH enzymes have been investigated as anti-cancer therapeutics (Lodola et al. 2015). However, after poor outcomes of important clinical studies on healthy human volunteers, the trials have had very stringent conditions, limiting further advances in the field. MAGL has also been explored as an option, as it is over-expressed in some cancer types as breast, ovarian, and melanoma. It was considered that its inhibition might contribute to lower cancer cell migration and aggressiveness (Granchi et al. 2017). MAGL is crucial for lipid metabolism and might as such also be useful as a target for activation of lipid signaling pathways important for cancer cell spread, survival, and growth. It is an ECS enzyme with the highest number of patented uses for many therapeutic uses, including anti-cancer treatment.

The Role of ECS in Response to Standard Anti-Cancer Treatment

During many experimental assessments of the effect of various ECS components on cancer growth, spread, and signaling, it has been reported that they tend to interact with standard anti-cancer therapies. This effect needs to be considered in each setting when a concurrent treatment is ongoing, as there might be a change in the cytotoxic mechanisms that was expected to occur.

A clinical study exploring the potential beneficial effect of adding cannabinoids to standard anti-cancer treatment for recurrent glioblastoma multiforme recently showed some promising results (Twelves et al. 2021). The authors evaluated the safety profile and effectiveness of nabiximols oromucosal spray given concurrently with dose-intense temozolomide (DIT) after first recurrence of GBM. At 1 year of treatment, survival rate was 83% for nabiximols-treated and 44% for placebo-treated patients, and nabiximols showed acceptable safety and tolerability. No apparent disruptive effect of nabiximols was detected on temozolomide pharmacokinetics. This positive result of a phase 1b study supports further efforts to design an adequately powered randomized controlled trial and evaluate survival rates of this new potential treatment combination. For some cannabinoids as THC and CBD, it has been show that they promote the cytotoxic activity of chemotherapeutics as vinblastine, cytarabine, mitoxantrone, doxorubicin, vincristine, mitoxantrone, carmustine, carfilzomib, temozolomide, bortezomib, carfilzomib, and cisplatin (Ramer and Hinz 2017). In early 2022, a first large-scale phase II trial in the UK will assess the potential of combining Sativex (an oral spray of THC and CBD) to standard chemotherapeutic regimen temozolomide to has benefit in patients with recurrent glioblastoma (ARISTOCRAT study). The trail aims to assess whether concurrent treatment with Sativex and chemotherapy will prolong the overall survival or delay disease progression, i.e., prolong the progression-free survival and/or improve patients' overall quality of life.

Future areas of research focusing on ECS-related anti-cancer therapeutics will most probably be exploring their usability in joint scenarios with chemo-, targeted, and immunotherapy drugs, considering all the specificities of the cancer subtype in question. Many standard anti-cancer protocols, although effective in combating cancer, have significant adverse effects, thus lowering the patients' quality of life.

Many plant derivatives and components, as well as cannabinoids, have been proposed as beneficial in this setting, as supplementary or alternative drugs, aiding patients to achieve a maximum anti-cancer response while protecting their performance status (Srdic-Rajic et al. 2016, 2017; Fraguas-Sánchez and Torres-Suárez 2018). In the case of lung cancer, which is a model subtype for the rising success of targeted therapies worldwide (Cavic et al. 2016; Jankovic et al. 2019), there is also a problem of acquired resistance that involves the epithelial to mesenchymal transition (Milian et al. 2020). Complementary drugs based on ECS components might also prove useful for restoring the epithelial phenotype contributing to prolonged lung cancer patient survival. The options for their administration and routes of absorption will also need to be further evaluated in detail before their use in cancer therapy. As their application for other human disorders and palliative medicine is already well described, drug repurposing protocols might be employed to speed up the necessary clinical processes of evaluation, as has been proposed for other therapeutics (Grahovac et al. 2019). The era of avoiding ECS components in anti-cancer treatment due to ethical and legal stigmas that accompanied the abuse of exogenous cannabinoids seems to slowly be coming to an end. The risk-to-benefit ration of their use in any clinical trial will need to be carefully evaluated, as is the case with any new drug family that is introduced in pharmacological testing on larger patient populations.

Conclusions

Although many aspects of the role of the ECS in the oncological setting have been rigorously evaluated in preclinical studies, its clinical utility remains the topic of vivid scientific debate. The ECS is vital for the maintenance of physiological homeostasis, and its deregulation has great significance for the occurrence of cancer, its progression, prognosis, interaction with standard anti-cancer therapeutics, and anti-cancer treatment. Basic research has yielded scientific evidence that contributed to the initiation of clinically oriented studies involving repurposing of ECS-based drugs from their primary use in pain alleviation. However, legal and ethical issues that accompany the use of cannabinoids in anti-cancer treatment seem to still precede its value. The scientific community is therefore posed before a difficult task to address all the potential non-scientific pitfalls while providing specific grades of recommendation and levels of evidence for future ECS-based anti-cancer therapeutics.

Acknowledgments This work was supported by Grant No. PID2020-113938RB-I00 (funded by MCIN/AEI/10.13039/501100011033) and the Ministry of Education, Science, and Technological Development of the Republic of Serbia (451-03-68/2022-14/200043).

Author Contributions EM and MC: data curation, conceptualization, writing – original draft preparation, writing – review and editing. EIC: conceptualization, writing – review and editing, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

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