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Changes in the Endocannabinoid System May Give Insight into new and Effective Treatments for Cancer

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Abstract

The endocannabinoid system comprises specific cannabinoid receptors such as Cb1 and Cb2, the endogenous ligands (anandamide and 2-arachidonyl glycerol among others) and the proteins responsible for their synthesis and degradation. This system has become the focus of research in recent years because of its potential therapeutic value several disease states. The following review describes our current knowledge of the changes that occur in the endocannabinoid system during carcinogenesis and then focuses on the effects of anandamide on various aspects of the carcinogenic process such as growth, migration, and angiogenesis in tumors from various origins.

I. Introduction

Marijuana and its derivatives have been used in medicine for centuries, however, it was not until the isolation of the psychoactive component of Cannabis sativa (Δ^9-tetrahydrocannabinol; Δ^9-THC) and the subsequent discovery of the endogenous cannabinoid signaling system that research into the therapeutic value of this system reemerged. Ongoing research is determining that regulation of the endocannabinoid system may be effective in the treatment of pain (Calignano *et al.*, 1998; Manzanares *et al.*, 1999), glaucoma (Voth and Schwartz, 1997), and neurodegenerative disorders such as Parkinson's disease (Piomelli *et al.*, 2000) and multiple sclerosis (Baker *et al.*, 2000). In addition, cannabinoids might be effective anti-tumoral agents because of their ability to inhibit the growth of various types of cancer cell lines in culture (De Petrocellis *et al.*, 1998; Ruiz *et al.*, 1999; Sanchez *et al.*, 1998, 2001) and in laboratory animals (Galve-Roperh *et al.*, 2000).

The endogenous cannabinoid system (endocannabinoid) consists of the cannabinoid receptors, their endogenous ligands (endocannabinoids) and the proteins for their synthesis and inactivation (Bisogno *et al.*, 2005). The cannabinoid receptors are seven-transmembrane-domain proteins coupled to $G_{i/o}$ type of G-proteins (Bisogno *et al.*, 2005). To date, there are two definitive cannabinoid receptors, Cb1 and Cb2, as well as a putative involvement of the vanilloid receptor VR1. More recently, the orphan receptor GPR55 was shown to function as a novel cannabinoid receptor (Ryberg *et al.*, 2007). Cb1 receptors are found predominantly in the central nervous system, but also in most peripheral tissues including immune cells, the reproductive system, the gastrointestinal tract and the lungs (Devane *et al.*, 1988; Matsuda *et al.*, 1990; Munro *et al.*, 1993). Cb2 receptors are found predominantly in the immune system, that is, in tonsils, spleen, macrophages, and lymphocytes (Devane *et al.*, 1988; Matsuda *et al.*, 1990; Munro *et al.*, 1993).

To date, many endocannabinoids have been identified with varying affinities for the receptors and all of which are lipid molecules. Anandamide (AEA) was the first endogenous ligand to be identified (Devane *et al.*, 1988), which acts as a partial Cb1 agonist and weak Cb2 agonist. It has also been shown to activate the GPR55 (Ryberg *et al.*, 2007). While the physiological roles of many of the other ligands have not yet been fully clarified, AEA has been implicated in a wide variety of physiological and pathological processes.

Currently, there are two biosynthesis pathways for AEA. The first is via the remodeling of an existing membrane phosphoglyceride, that is, the calcium-dependent *N*-transacylation of phosphotidylethanolamine with arachidonic acid to form *N*-arachidonyl-phosphatidyl-ethanolamine, which is then hydrolyzed to AEA (Bisogno *et al.*, 2005; Di Marzo and Deutsch, 1998). The enzyme responsible for the catalysis of this pathway is phospholipase D (Bisogno *et al.*, 2005). The second pathway is via the *de novo* synthesis of AEA from arachidonic acid and ethanolamine by the enzyme anandamide amidohydrolase catalyzing the reverse reaction from high levels of ethanolamine (Di Marzo and Deutsch, 1998). After synthesis, AEA is rapidly inactivated via a tightly controlled series of events involving sequestration by cells and enzymatic hydrolysis. The mechanism of AEA uptake is largely unknown with data suggesting that it is via passive diffusion and others suggesting that it is through the presence of an active transporter (Glaser *et al.*, 2005). Regardless of the mechanism, this uptake is a rapid event with a half-life of approximately 2.5 min (Di Marzo and Deutsch, 1998). After uptake, AEA is hydrolyzed and degraded by the enzyme anandamide amidohydrolase (also called fatty acid amide hydrolase or FAAH) (Di Marzo and Deutsch, 1998).

II. Changes in the Endocannabinoid System in Cancer

Evidence suggests that the endocannabinoid system may be dysregulated in a number of cancers (summarized in Table 18.1). Indeed, both AEA and 2-arachidonyl glycerol (2-AG) have been shown to be increased in human colorectal adenomatous polyps and carcinomas compared to normal colorectal mucosa (Ligresti *et al.*, 2003), suggesting that these endocannabinoids increase when passing from normal to transformed mucosa. No consistent differences were observed in the expression levels of Cb1, Cb2, or FAAH as assessed by both RT-PCR and immunoblotting between normal and colorectal cancer tissue (Ligresti *et al.*, 2003). Similarly, AEA levels were enhanced by 17-fold in glioblastomas whereas meningiomas were characterized by a massively enhanced level of 2-AG (Petersen *et al.*, 2005). Coupled with these changes was a 60% reduction in the enzyme activities of the AEA degradation enzymes, *N*-acylphosphotidylethanolamine-hydrolyzing phospholipase D and fatty acid amide hydrolase in the glioblastoma tissue and an enhanced *in vitro* conversion of phosphotidyl choline to monoacyl glycerol in the meningioma tissue (Petersen *et al.*, 2005). Similarly, the levels of AEA and 2-AG were found to be increased in human pituitary adenomas compared to normal pituitary gland (Pagotto *et al.*, 2001). Moreover, endocannabinoid content in the different pituitary adenomas correlated with the presence of Cb1, being elevated in the tumoral samples positive for Cb1 and lower in the samples in which no or low levels of Cb1 were found (Pagotto *et al.*, 2001). In another study, the levels of AEA were found to differ depending upon the source of the tumor (Schmid *et al.*, 2002). For example, AEA was increased in prostate carcinoma, endometrial sarcoma, and thigh histiocytoma, with no change in ileum lymphoma, and bladder carcinoma and a significant decrease in stomach carcinoma (Schmid *et al.*, 2002).

Furthermore, the cannabinoid receptor system has also been shown to be altered during the carcinogenic process. Indeed, in Mantle cell lymphoma and prostate cancer cells, both Cb1 and Cb2 are upregulated compared to nonmalignant tissue (Ek *et al.*, 2002; Islam *et al.*, 2003; Sarfaraz *et al.*, 2005), whereas in acute myeloid leukemia, only Cb2 is upregulated (Alberich Jorda *et al.*, 2004). A differential effect on receptor expression is shown in breast

cancer, with a marked suppression of Cb1 and an increased Cb2 expression (Caffarel *et al.*, 2006). In another study using human brain tissue, the expression of VR1 was aberrantly expressed in glioma brain tumors compared to nonmalignant brain tissue sampled from epileptic patients undergoing surgery (Contassot *et al.*, 2004b).

Dysregulation of the endocannabinoid system still needs to be examined in a number of other cancer types and the implications of a dysregulated endocannabinoid system on malignant transformation and tumor progression remains to be addressed.

III. Antiproliferative Effects of Anandamide

There is a large volume of data indicating antiproliferative effects of AEA on various cancer types via a number or receptor-dependent and receptor-independent mechanisms. A schematic diagram of the proposed mechanisms is depicted in Fig. 18.1.

A. Receptor mediated effects

Cb1—AEA has been shown to decrease the proliferation of breast cancer cells *in vitro* by decreasing the levels of the long form of the prolactin receptor (De Petrocellis *et al.*, 1998) and inhibiting the nerve growth factor (NGF)-mediated proliferation by decreasing the expression of the *trk* NGF receptor (Melck *et al.*, 2000). This was shown to be via a Cb1-dependent mechanism and involves the activation of the cAMP/protein kinase A pathway and subsequent activation of the mitogen-activated protein kinase (MAPK) pathway (Melck *et al.*, 1999). Furthermore, these growth suppressing effects of AEA are not due to toxicity or apoptosis, but by cell cycle arrest in the S phase which was correlated with an activation of Chk1 (an S phase checkpoint kinase), and a suppression of Cdk2 activity (Laezza *et al.*, 2006).

Similar antiproliferative and apoptotic effects of AEA were found in human prostate cancer cell lines (Mimeault *et al.*, 2003), which were also via the Cb1-dependent downregulation of growth factor signaling (Mimeault *et al.*, 2003). Specifically, AEA decreased the expression of the epidermal growth factor receptor (EGFR) in several prostate cancer cell lines which was associated with a G_1 arrest and cell death (Mimeault *et al.*, 2003). Furthermore, this effect could be partially blocked by acidic ceramide inhibitors indicating that AEA might be induced via the cellular ceramide production (Mimeault *et al.*, 2003).

Cb2—In contrast, the antiproliferative actions of AEA on glioma and asytocytoma cells are via the activation of the Cb2 receptor (Sanchez *et al.*, 2001). Treatment of mice inoculated with human glioma tumor cells or human astrocytoma cells with the specific Cb2 agonist, JWH-133 completely blocked the tumor growth *in vivo* (Sanchez *et al.*, 2001), and was, once again, associated with increased *de novo* ceramide synthesis *in vitro* (Sanchez *et al.*, 2001). Furthermore, targeting the Cb2 receptor has been suggested as a therapy to treat malignant lymphoblastic diseases (McKallip *et al.*, 2002). In these types of cancers, AEA induced apoptosis *in vitro* and *in vivo* and it is suggested that because the Cb2 agonists lack psychotropic effects, targeting of the Cb2 receptor would be preferential to targeting the Cb1 receptor in cancers of immune origin (McKallip *et al.*, 2002). Another study demonstrated that interleukin-12 treatment and/or overexpression in thyroid carcinoma cells leads to an increase in Cb2 expression and that either overexpression of interleukin-12 or Cb2 resulted in tumor regression and increased sensitivity to chemotherapy (Shi *et al.*, 2008). Taken together, these data indicate that targeting Cb2 may be of therapeutic value in certain tumor types and warrants further investigation.

VR1—The actions of AEA on VR1 were shown to be antiproliferative in glioma cells (Contassot *et al.*, 2004b) and in uterine cervix cancer cells (Contassot *et al.*, 2004a). In both of these cell types, the VR1 was aberrantly expressed in the tumor cells and tissue compared

to their nonmalignant counterparts (Contassot *et al.*, 2004a,b). Furthermore, stimulation of Cb1 or Cb2 was protective against the VR1-mediated antiproliferative effects of AEA (Contassot *et al.*, 2004a,b).

Combination of receptors—In addition to the above-mentioned findings, studies have shown an antiproliferative/growth suppressive effect of AEA that could not be attributable to just one receptor. Using rat C6 glioma cells, AEA was shown to have growth suppressing effects that were time and dose-dependent (Jacobsson *et al.*, 2001). These effects could be partially blocked by the Cb1, Cb2, and VR1 antagonists alone, but was completely attenuated when the three receptor antagonists were added in combination (Jacobsson *et al.*, 2001). In addition, in osteosarcoma cells, AEA induced apoptosis by increasing intracellular calcium levels, activation of p38 MAPK and subsequent activation of caspase 3 (Hsu *et al.*, 2007). Unfortunately, the authors do not address the issue of receptor-dependence in this study, therefore for the purpose of this review we will assume potential involvement of all three receptors as none can be ruled out.

B. Receptor-independent effects

Cannabinoid receptor-independent actions of AEA have been described in several tumor cell types (DeMorrow *et al.*, 2007; Hinz *et al.*, 2004a,b; Patsos *et al.*, 2005; Ramer *et al.*, 2001). Furthermore, most of these effects are via the actions of AEA at the cell membrane (DeMorrow *et al.*, 2007; Hinz *et al.*, 2004a,b; Ramer *et al.*, 2001). Treatment of human neuroglioma cells with the stable analogue of AEA (Met-AEA) resulted in the induction of apoptosis (Hinz *et al.*, 2004a,b; Ramer *et al.*, 2001). This effect could not be blocked by the coadministration of antagonists of the Cb1, Cb2, or VR1 receptors, nor the $G_{i/o}$ protein inactivator pertussis toxin (Ramer *et al.*, 2001). Coupled with the induction of apoptosis by Met-AEA, there was an increased synthesis of ceramide, expression of cyclooxygenase-2 (Cox-2) and subsequent prostaglandin E2 synthesis via a mechanism involving p38 and p42/44 MAPK activation (Ramer *et al.*, 2001). Specific Cox-2 inhibitors, such as celecoxib and diclofenac, or the specific silencing of Cox-2 expression with small-interfering RNA blocked the Met-AEA-induced apoptosis (Hinz *et al.*, 2004b). Furthermore, the lipid raft disruptor methyl-beta-cyclodextrin blocked the Met-AEA-induced effects of ceramide synthesis, phosphorylation of p38 and p42/44 MAPK, expression of Cox-2, and subsequent prostaglandin E2 synthesis (Hinz *et al.*, 2004a). Together, these data suggest that Met-AEA, via lipid raft-mediated events, induces apoptosis of neuroglioma cells. A similar effect of AEA was observed in colorectal cancer cells (Patsos *et al.*, 2005). Treatment of these cells with AEA increased the expression of Cox-2 and induced a subsequent cell death pathway (Patsos *et al.*, 2005). Interestingly, inhibition of FAAH potentiated this cell death, suggesting that AEA-induced cell death was mediated via the metabolism of AEA by Cox-2 rather than through the classical AEA degradation pathway (Patsos *et al.*, 2005).

More recently, we have shown that AEA can induce growth-suppressive/pro-apoptotic effects in cholangiocarcinoma cells which could not be blocked by any Cb1, Cb2, or VR1 antagonists nor the $G_{i/o}$ protein inactivator pertussis toxin (DeMorrow *et al.*, 2007). This is via the stabilization of the lipid raft structures within the plasma membrane, the increased production of ceramide, and the subsequent recruitment of the death receptor complex components into the lipid raft structures (DeMorrow *et al.*, 2007). Interestingly, the other more prevalent endocannabinoid, 2-AG, had growth-promoting effects, which were shown to be via the complete disruption of the lipid raft structure (DeMorrow *et al.*, 2007), an event which has previously been shown to result in growth-promoting effects in other cell types (Lambert *et al.*, 2006; Mathay *et al.*, 2008).

These receptor-independent mechanisms are summarized in Fig. 18.2. These so-called "receptor-independent" effects of AEA must be taken with a note of caution. Most of these studies were performed prior to the identification of GPR55 as a putative cannabinoid receptor. Therefore, the possibility that AEA is exerting its effects through GPR55 or some other, as yet unidentified cannabinoid receptor cannot be ruled out.

IV. Effects of AEA on Migration, Invasion, and Angiogenesis

The acquisition of metastatic abilities by cancer cells often leads to clinically incurable disease. Metastasis consists of a series of sequential steps including detachment of cells from the primary tumor, survival of the cells in circulation, arrest in a new organ, initiation and maintenance of growth in the new tissue, and vascularization of the metastatic tumor (Fidler, 2002). AEA has been shown to have a regulatory role at each of these stages in the metastatic process (Fig. 18.3). Firstly, AEA was shown to be an endogenous inhibitor of migration of both colon carcinoma cells and nonmalignant T lymphocytes *in vitro* (Joseph *et al.*, 2004). There was a differential mechanism involved in the regulation of the tumor versus immune cells, that is, tumor cell migration could be stimulated by specific agonists for Cb1 receptor, whereas the immune cell migration was inhibited by a Cb2-dependent mechanism (Joseph *et al.*, 2004). Furthermore, in an *in vivo* model of metastatic spreading using breast cancer cell lines, the AEA analogue, met-AEA significantly reduced the number and dimension of metastatic nodes, an effect that was inhibited by specific Cb1 receptor antagonists (Grimaldi *et al.*, 2006). Molecular changes in the organization and distribution of cytoskeleton proteins are necessary for focal adhesion; cell motility and cell invasion were then assessed. No changes in expression of any of the integrins were detected after Met-AEA treatment, however, there was a marked decrease in the phosphorylation of focal adhesion kinase and src, both of which are normally localized to the focal adhesions and are involved in the metastatic formation and development (Grimaldi *et al.*, 2006).

In order for a migrating cancer cell to then invade another organ, the existing extracellular matrix components (e.g., collagens and proteoglycans) must be broken down and hence the rigid architecture of the target organ must be compromised. Matrix metalloproteinases (MMPs) are emerging as a family of enzymes that exerts important functions during tumor invasion (Curran and Murray, 2000; Stamenkovic, 2000). Tissue inhibitors of MMPs (TIMPs), and in particular TIMP-1, have also been shown to inhibit the proteolytic activity of MMPs and suppress vascular tumor growth and angiogenesis in xenograft animal models (Zacchigna *et al.*, 2004). Furthermore, there appears to be a correlation between high cancer invasiveness and decreased TIMP-1 expression (Chan *et al.*, 2005; Khokha *et al.*, 1989). Recently, the effects of AEA on MMP and TIMP expression were evaluated in various cancer cell types. Using a cervical cancer cell line, Met-AEA as well as Δ^9-THC, inhibited the invasive properties of these cells via the increased expression of TIMP-1 (Ramer and Hinz, 2008). The effects of the cannabinoids on invasion and TIMP-1 expression were inhibited by the pretreatment of the cells with Cb1 and Cb2 antagonists as well as specific inhibitors of the p38 and p42/44 MAPkinases (Ramer and Hinz, 2008). This effect of cannabinoids on TIMP-1 expression was mimicked by treatment of glioma cells with Δ^9-THC (Blazquez *et al.*, 2008a).

Conversely, in glioma cells, cannabinoid treatment selectively decreased the expression of MMP-2 via a Cb2-dependent mechanism and requiring the synthesis of ceramide (Blazquez *et al.*, 2008b). By manipulating the levels of MMP-2 expression by siRNA and cDNA overexpression, the authors were able to show that the decrease in MMP-2 expression was critical for the cannabinoid-mediated inhibition of cell invasion (Blazquez *et al.*, 2008b).

The last aspect of the metastatic process that is regulated by cannabinoids is angiogenesis. Met-AEA inhibited the basic fibroblast growth factor-stimulated endothelial cell proliferation and

induced apoptosis in a Cb1-dependent manner (Pisanti *et al.*, 2007). Furthermore, Met-AEA was able to inhibit bidimensional capillary-like tube formation and tumor-induced angiogenesis in a three-dimensional model of endothelial and thyroid tumor cell spheroid cocultures (Pisanti *et al.*, 2007). In support of this, we have shown that AEA treatment of an *in vivo* xenograft model of cholangiocarcinoma also markedly inhibits the expression of members of the vascular endothelial growth factor family (DeMorrow *et al.*, 2008), which are key regulators of both normal and abnormal angiogenesis (Ferrara, 2005; Ferrara and Kerbel, 2005).

Together, these data suggest that cannabinoids, and in particular anandamide, may be key endogenous inhibitors of various stages in metastatic processes, including migration, invasion, and angiogenesis. This further supports the notion that drugs directed at regulating the endocannabinoid system may prove to be valuable tools in the fight against various cancers.

V. Targeting Degradation Enzymes of Cannabinoids as an Anticancer Therapy

As mentioned previously, the degradation of endocannabinoids is an active and rapid process. Therefore, blocking the degradation pathway may enhance the antiproliferative effects of AEA and have beneficial effects in cancer treatment. Indeed, treatment of human breast cancer cells *in vitro* with palmitoylethanolamide enhances the antiproliferative effects of AEA (Di Marzo *et al.*, 2001). This agent was shown to reduce the expression of FAAH up to 30–40% thereby allowing the accumulation of AEA and increasing its antiproliferative effects (Di Marzo *et al.*, 2001). In addition, treatment of athymic mice with thyroid tumor xenografts with VDM-11 (a selective inhibitor of endocannabinoid cellular reuptake) or arachidonyl-serotonin (a selective blocker of endocannabinoid hydrolysis) increased the intratumoral levels of anandamide and significantly decreased tumor volume (Bifulco *et al.*, 2004). The antiproliferative actions of these agents could be only partly inhibited by the pretreatment of the Cb1 receptor antagonist, suggesting that endocannabinoids tonically control tumor growth *in vivo* by both Cb1-mediated and non-Cb1-mediated mechanisms (Bifulco *et al.*, 2004). Regardless of the molecular mechanism by which anandamide and other endocannbinoids regulate tumor growth, inhibitors of their inactivation might be useful for the development of novel anticancer drugs (Bifulco *et al.*, 2004).

VI. Tumor Promoting Effects of Anandamide

The evidence supporting growth-promoting effect of AEA in tumors is pallid in comparison to the antitumoral effects described above. There is a greater volume of research indicating that the structurally similar, plant-derived cannabinoid, Δ^9-THC stimulates growth in a number of cancer cell lines via Cb1 and Cb2 receptor-independent mechanisms (McKallip *et al.*, 2005; Takeda *et al.*, 2008). However, several cannabinoids, including AEA, have been shown to accelerate proliferation via the transactivation of the EGFR in a TACE/ADAM17 metalloprotease-dependent manner (Hart *et al.*, 2004). This effect was observed in several cell lines from various origins including lung cancer, squamous cell carcinoma, bladder carcinoma, glioblastoma, astrocytoma, and kidney cancer (Hart *et al.*, 2004). The cannabinoid-induced activation of the EGFR leads to the subsequent phosphorylation and activation of the adaptor protein Src homology 2 domain-containing (shc), and downstream activation of the ERK1/2 and Akt/PKB pathways (Hart *et al.*, 2004). Thus, the cross-communication of cannabinoid receptors and EGFR may provide an explanation as to how cannabinoids may stimulate cancer cell proliferation (Hart *et al.*, 2004).

In addition, the stable analogue of AEA, methanandamide, has a mitogenic effect on an androgen-dependent prostate cancer cell line that could be blocked by antagonists for either

the Cb1 or Cb2 receptors as well as by the PI-3kinase inhibitor (Sanchez *et al.*, 2003). The downstream consequence of activation of the endocannabinoid system was an increase in the expression of the androgen receptor, which is directly linked to the growth of these cells (Sanchez *et al.*, 2003).

VII. Conclusions

In conclusion, the endocannabinoid system exerts a myriad of effects on tumor cell growth, progression, angiogenesis, and migration. With a notable few exceptions, targeting the endocannabinoid system with agents that activate cannabinoid receptors or increase the endogenous levels of AEA may prove to have therapeutic benefit in the treatment of various cancers. Further studies into the downstream consequences of AEA treatment are required and may illuminate other potential therapeutic targets.

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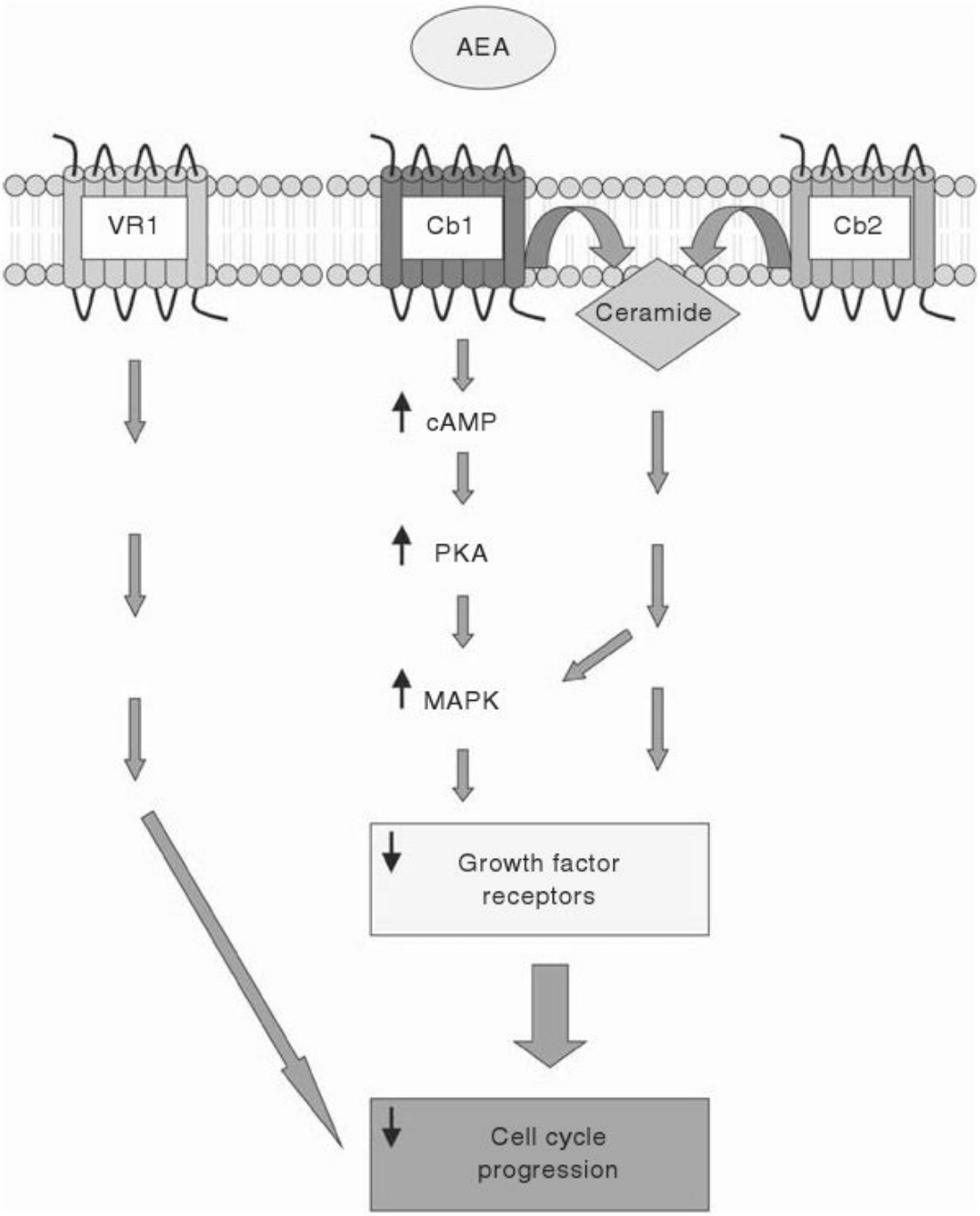

Figure 18.1.
Schematic diagram of the cannabinoid receptor-dependent mechanisms whereby AEA leads to tumor growth suppression and/or cell death. AEA may act via the Cb1 receptor to activate the cAMP/PKA/MAPK pathway or to increase the production of ceramide. These effects ultimately result in a decrease in the expression of various growth factor receptors and decrease cell cycle progression. Alternatively, AEA may activate either Cb2 or VR1 to elicit a similar response, although the mechanism by which this occurs is not clear.

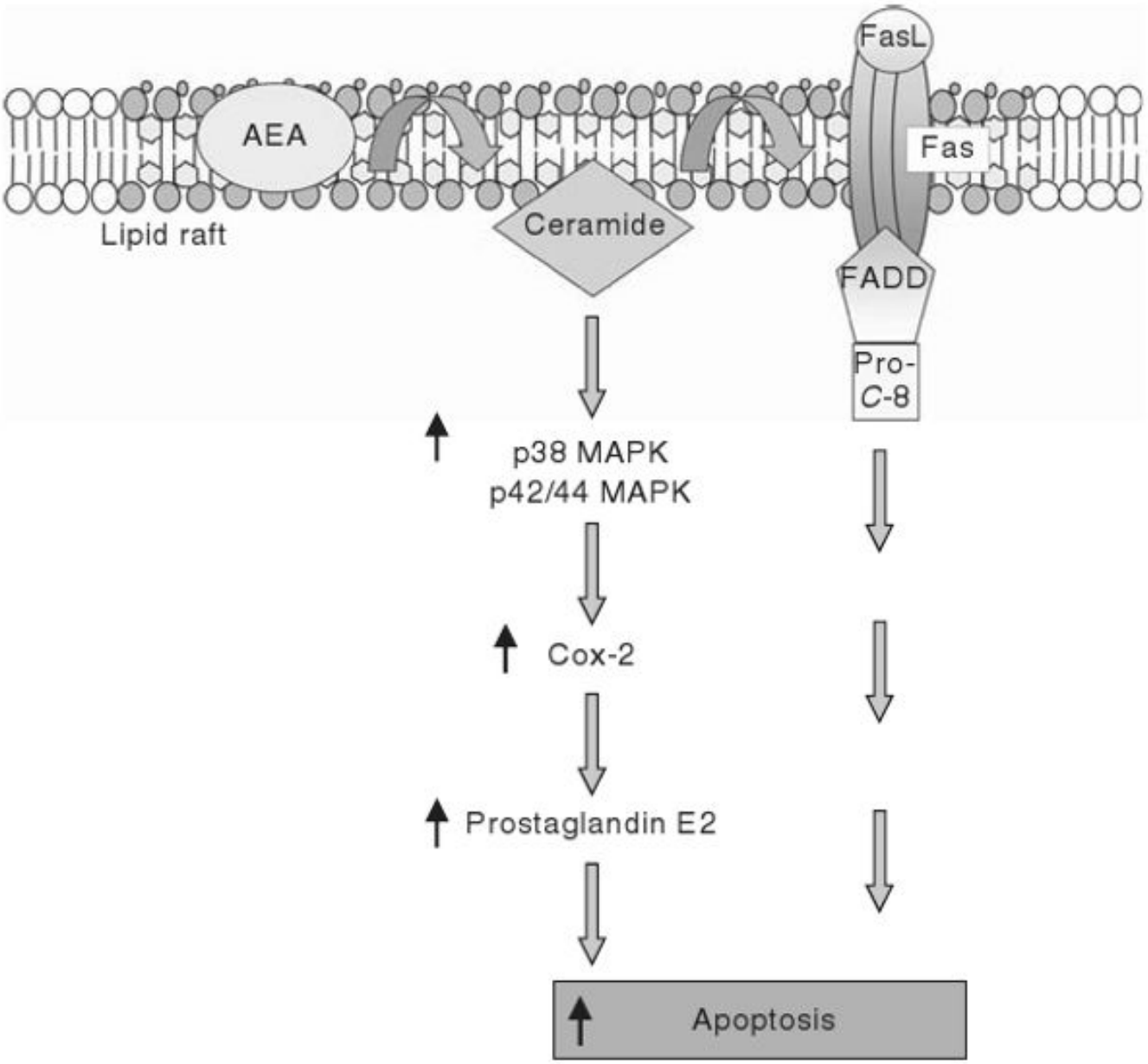

Figure 18.2.
Schematic diagram of the cannabinoid receptor-independent mechanisms whereby AEA induces apoptosis. AEA stabilizes the lipid raft microdomains at the plasma membrane and increases ceramide production. This then has an effect on Cox-2 expression and prostaglandin E2 production with a subsequent increase in apoptosis. Alternatively, AEA-induced ceramide production facilitates the Fas/FasL death receptor complex within the lipid raft structure, which ultimately results in increased apoptosis.

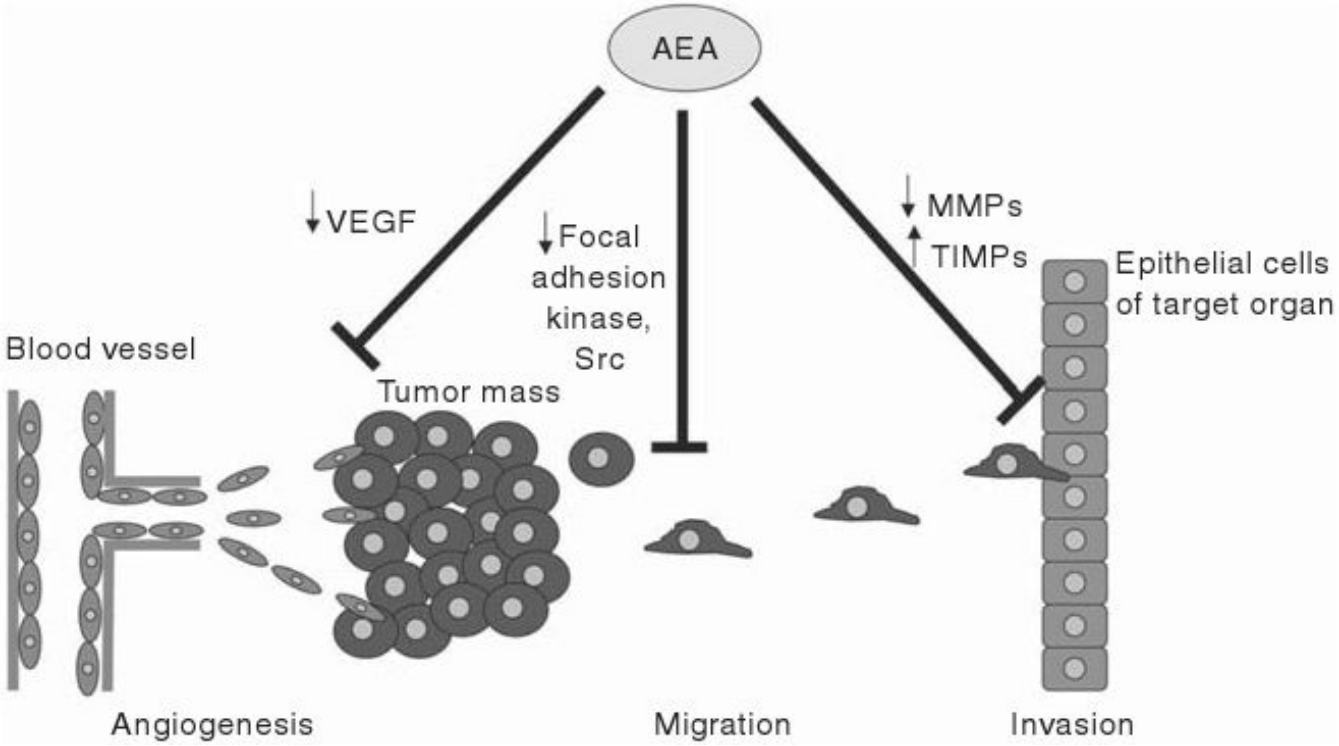

Figure 18.3.
AEA inhibits other aspects of tumorigenesis such as angiogenesis, tumor cell migration, and tumor invasion. AEA inhibits angiogenesis via a decrease in VEGF expression, whereas the decrease in migration is thought to be via a decrease in the activation of focal adhesion kinases and src kinase, both of which are thought to be involved in cell migration and metastasis. Lastly, AEA inhibits tumor cell invasion by decreasing the expression of proteins responsible for breaking down the extracellular matrix of the target organ, such as MMPs and increasing the expression of the tissue inhibitors of MMPs.

Table 18.1

Changes in the endocannabinoid system in various types of human cancer

Cancer type	***In vivo*/*in vitro***	**Changes in the endocannabinoid system**	**References**
Colorectal	*In vivo*	AEA ↑, 2-AG ↑	Ligresti *et al.* (2003)
Glioblastomas	*In vivo*	AEA ↑, FAAH ↓, Phopholipase D ↓, VR1 ↑	Contassot *et al.* (2004b) and Petersen *et al.* (2005)
Meningioma	*In vivo*	2-AG ↑	Petersen *et al.* (2005)
Pituitary adenomas		AEA ↑, 2-AG ↑, Cb1 ↑	Pagotto *et al.* (2001)
Prostate carcinoma	*In vivo*	AEA ↑, Cb1 ↑, Cb2 ↑	Schmid *et al.* (2002) and Sarfaraz *et al.* (2005)
Endometrial sarcoma	*In vivo*	AEA ↑	Schmid *et al.* (2002)
Thigh histiocytoma	*In vivo*	AEA ↑	Schmid *et al.* (2002)
Stomach carcinoma	*In vivo*	AEA ↓	Schmid *et al.* (2002)
Mantle cell lymphoma	*In vivo*	Cb1 ↑, Cb2 ↑	Ek *et al.* (2002) and Islam *et al.* (2003)
Acute myeloid leukemia	*In vitro*	Cb2 ↑	Alberich Jorda *et al.* (2004)
Breast cancer	*In vivo*	Cb1 ↓, Cb2 ↓	Caffarel *et al.* (2006)