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Antitumorigenic Effects of Cannabinoids beyond Apoptosis

Nadine Freimuth, Robert Ramer, and Burkhard Hinz

Institute of Toxicology and Pharmacology, University of Rostock, Rostock, Germany

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ABSTRACT

According to the World Health Organization, the cases of death caused by cancer will have been doubled until the year 2030. By 2010, cancer is expected to be the number one cause of death. Therefore, it is necessary to explore novel approaches for the treatment of cancer. Over past years, the antitumorigenic effects of cannabinoids have emerged as an exciting field in cancer research. Apart from their proapoptotic and antiproliferative action, recent research has shown that cannabinoids may likewise affect tumor cell angiogenesis, migration, invasion, adhesion, and metastasization. This review will summarize the data concerning the influence of cannabinoids on these locomotive processes beyond modulation of cancer cell apoptosis and proliferation. The findings discussed here provide a new perspective on the antitumorigenic potential of cannabinoids.

Cannabinoids are currently used in cancer patients to palliate wasting, emesis, and pain. In addition, evidence has been accumulated over the last decade to suggest that these compounds could also be useful for the inhibition of tumor cell growth by modulating several survival pathways. Although anticancer effects of cannabinoids were shown as early as 1975 in Lewis lung carcinoma (Munson et al., 1975), interest in anticarcinogenic properties of these compounds was even renewed after the discovery of the cannabinoid system and the cloning of specific $G_{i/o}$-coupled cannabinoid receptors CB_1 and CB_2 (De Petrocellis et al., 1998; for review, see also Howlett et al., 2002; Abood, 2005). Although the majority of effects of cannabinoids are mediated via CB_1 and CB_2, the transient receptor potential vanilloid type 1 (TRPV1) has been described as an additional receptor target for several cannabinoids (Zygmunt et al., 1999; Costa et al., 2004; Ligresti et al., 2006). Finally, there are also various cannabinoid effects that have been associated with molecular events independent of either CB_1/CB_2 or TRPV1 activation (Ruiz et al., 1999; Hinz et al., 2004; Vaccani et al., 2005; Fogli et al., 2006).

The first comprehensive approach to clarify the involvement of cannabinoid receptors in the antitumorigenic properties of cannabinoids was achieved by Galve-Roperh et al. (2000) using a xenograft rodent model. Meanwhile, cannabinoid administration to animals has been shown to induce the regression of a broad array of cancer types, such as gliomas (Galve-Roperh et al., 2000; Sánchez et al., 2001), thyroid epitheliomas (Bifulco et al., 2001), lymphomas (McKallip et al., 2002), and skin carcinomas (Casanova et al., 2003). Moreover, several studies confirmed proapoptotic and antiproliferative effects of cannabinoids in different cancer cells by mechanisms involving, for instance, de novo synthesis of ceramide (Galve-Roperh et al., 2000; Hinz et al., 2004) or

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ABBREVIATIONS: Akt, protein kinase B; AEA, anandamide; Ang-1/2, angiopoietin 1/2; 2-AG, 2-arachidonylglycerol; CBD, cannabidiol; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; DEA, docosatetraenylethanolamide; ERK 1/2, extracellular-signal regulated kinase 1/2; FAK, focal adhesion kinase; FRNK, FAK-related nonkinase; HIF-1α, hypoxia-inducible factor 1α; HUVEC, human umbilical vascular endothelial cells; Id-1/3, inhibitor of differentiation 1/3; IgSF CAMs, cell adhesion molecules of the immunoglobulin superfamily; IL-1, interleukin-1; MMP, matrix metalloproteinase; MMP-2/9, matrix metalloproteinase 2/9; MA, methanandamide; Met-F-AEA, met-fluoro-anandamide; NSCLC, non–small cell lung cancer; PlGF, placental growth factor; RHOA, Ras homolog gene family member A; RHOA-ROCK, RhoA/Rho-associated coiled coil-containing kinase; ROS, reactive oxygen species; Src, proto-oncogenic tyrosine kinase; THC, Δ^9-tetrahydrocannabinol; Tie-1, tyrosine kinase with immunoglobulin-like and EGF-like domains 1; TIMP-1, tissue inhibitor of metalloproteinases 1; TRPV1, transient receptor potential vanilloid 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

activation of mitogen-activated protein kinases (Galve-Roperh et al., 2000; Herrera et al., 2005). Furthermore, recent data support the hypothesis that cannabinoid receptors together with endogenously produced agonists contribute to an endogenous defense mechanism against tumorigenesis (Bifulco et al., 2004; Ligresti et al., 2003; Di Marzo et al., 2004; Kishimoto et al., 2005; Wang et al., 2008).

Apart from regulating tumor cell growth and apoptosis (for review, see Guzmán et al., 2002; Bifulco and Di Marzo, 2002), other antitumorigenic mechanisms of cannabinoids are currently emerging as a focus of research work. Therefore, the present review focuses on the impact of cannabinoids on tumor neovascularization, tumor cell migration, adhesion, invasion, and metastasization (see Fig. 1). Table 1 provides an overview of the cannabinoids mentioned in this review.

Impact of Cannabinoids on Tumor Neovascularization. In the early 1970s, Judah Folkman was the first to propose the idea that angiogenesis is a crucial event for solid tumors to grow beyond 1 to 2 mm^3 or to become metastatic (Folkman, 1971, 1972). In this context, cannabinoids were demonstrated to cause a lower vascular density of experimental tumors as assessed by the lower distribution of CD31-positive cells in experimental tumor xenografts from glioma, melanoma, and nonmelanoma skin cancer and lung tumor cells (Blázquez et al., 2003, 2006; Casanova et al., 2003; Preet et al., 2008). Met-fluoro-anandamide (Met-F-AEA), a metabolically stable analog of the endocannabinoid AEA, has been demonstrated to confer a reduction of sprout number as well as sprout length of endothelial cell spheroids, an inhibition of capillary-like tube formation in vitro and a suppression of angiogenesis in an in vivo chick chorioallantoic membrane assay (Pisanti et al., 2007). Furthermore, experimental tumors from animals treated with cannabinoids were shown to exert a vascular network that is small, undifferentiated, and impermeable (Blázquez et al., 2003) and make tumors appear paler compared with the respective controls (Portella et al., 2003). In fact, numerous cannabinoids that bind to CB_1 and/or CB_2 receptors, including WIN-55,212-2, HU-210, JWH-133, and Δ^9-tetrahydrocannabinol (THC), inhibit vascular endothelial cell survival and migration as part of their antiangiogenic action (Blázquez et al., 2003).

As first suggested by Blázquez et al. (2003) from the group of Manuel Guzmán, besides this direct inhibition of vascular endothelial cell migration and survival, the decrease of the expression of proangiogenic factors in the tumors may be likewise involved substantially in the antiangiogenic action of cannabinoids. Accordingly, several studies indicate an impact of cannabinoids on the expression of vascular

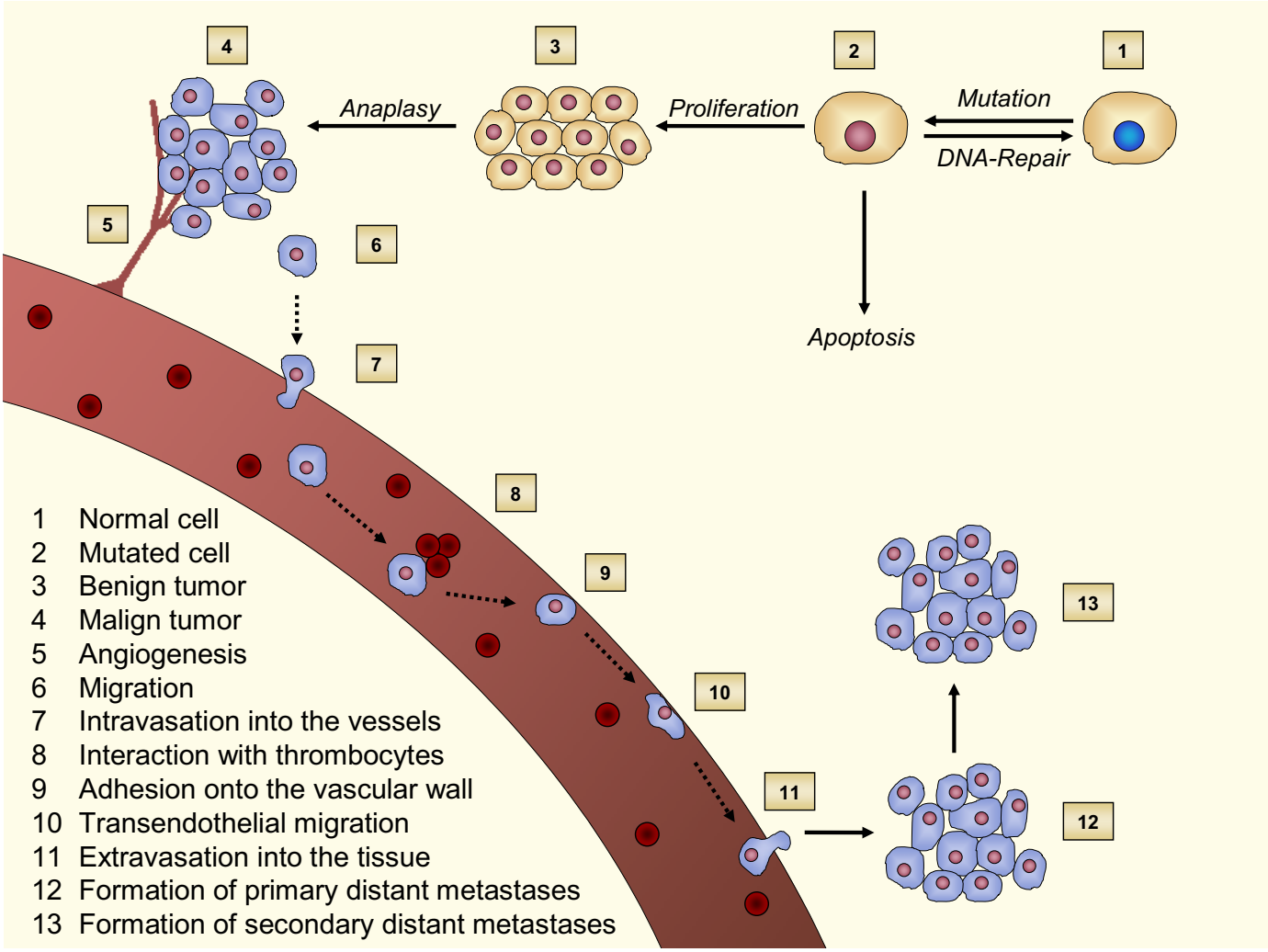

Fig. 1. Model of tumorigenesis. The figure illustrates the formation of an invasive tumor and its metastasization to a distant organ via a blood vessel.

TABLE 1
Overview on different cannabinoids and their receptor targets

Cannabinoid	Abbreviation	Chemical Name	Target(s)
Endocannabinoids			
Anandamide	AEA	*N*-(2-Hydroxyethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide	Nonselective agonist (CB_1>CB_2)
2-Arachidonylglycerol	2-AG	(5*Z*,8*Z*,11*Z*,14*Z*)-5,8,11,14-Eicosatetraenoic acid	Nonselective agonist (CB_1>CB_2)
Docosatetraenylethanolamide	DEA	*N*-(2-Hydroxyethyl)-7*Z*,10*Z*,13*Z*,16*Z*-docosatetraenamide	Selective CB_1 receptor agonist
Phytocannabinoids			
Cannabidiol	CBD	2-[(1*R*,6*R*)-3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol	Not fully clarified
Cannabinol	CBN	6,6,9-Trimethyl-3-pentyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol	Nonselective agonist (CB_1, CB_2)
Δ^9-Tetrahydrocannabinol	THC	(6*aR*,10*aR*)-6*a*,7,8,10*a*-Tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol	Nonselective agonist (CB_1, CB_2)
Synthetic cannabinoids			
CP 55,940	CP 55,940	(−)-*cis*-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol	Nonselective agonist (CB_1, CB_2)
HU-210	HU-210	(6*aR*)-*trans*-3-(1,1-Dimethylheptyl)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol	Nonselective agonist (CB_1, CB_2)
HU-331	HU-331	3-Hydroxy-2-[(1*R*,6*R*)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-2,5-cyclohexadiene-1,4-dione	Inhibition of topoisomerase II
JWH-133	JWH-133	(6*aR*,10*aR*)-3-(1,1-Dimethylbutyl)-6*a*,7,10,10*a*-tetrahydro −6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran	Selective CB_2 receptor agonist
Met-fluoro-anandamide	Met-F-AEA	2-Methyl-arachidonyl-2′-fluoro-ethylamide	Nonselective agonist (CB_1>CB_2)
Methanandamide	MA	(*R*)-*N*-(2-Hydroxy-1-methylethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide	Nonselective agonist (CB_1>CB_2)
WIN-55,212-2	WIN-55,212-2	(*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone	Nonselective agonist (CB_1, CB_2)

endothelial growth factor (VEGF), which is one of the major cancer cell-released chemoattractants in tumor neovascularization (for review, see Saia et al., 2007). Met-F-AEA was demonstrated to decrease levels of VEGF and VEGFR-1 in K-ras-transformed thyroid cells and in experimental tumors of nude mice xenografted with these cells (Portella et al., 2003). In line with these findings, analyses in skin carcinoma nude mouse models (Casanova et al., 2003) confirmed an inhibitory action of JWH-133 and WIN-55,212-2 on vascular hyperplasia, which was associated with a reduced mRNA expression of VEGF. Analysis of antimetastatic and antiangiogenic effects of THC on non–small cell lung cancer (NSCLC) cells also revealed a suppression of VEGF release (Preet et al., 2008). Using cDNA arrays, Blázquez et al. (2004) further provided evidence for a JWH-133-mediated decreased expression of proangiogenic key factors related to VEGF signaling in mouse gliomas, such as VEGF-A, VEGF-B, and hypoxia-inducible factor 1α (HIF-1α), the major transcription factor responsible for VEGF expression. In the same study, connective tissue growth factor and heme oxygenase-1, both genes known to be regulated by VEGF (Suzuma et al., 2000; Busolatti et al., 2004), as well as the VEGF-related factors [inhibitor of differentiation-3 (Id-3), midkine, and the angiopoietin receptor tyrosine kinase with immunoglobulin-like and epidermal growth factor (EGF)-like domains 1 (Tie-1)] could be demonstrated to be down-regulated by JWH-133. On the other hand, JWH-133 had an inductive effect on the expression of type I procollagen 1α chain (Blázquez et al., 2004), a matrix metalloproteinase (MMP) substrate related to matrix remodeling during angiogenesis (Seandel et al., 2001).

In vivo experiments furthermore demonstrated that JWH-133 and WIN-55,212-2 decrease mRNA levels and autophosphorylation activity of EGF receptor (EGFR) in skin tumors (Casanova et al., 2003). In the same study, cannabinoids diminished the expression of angiopoietin-2 (Ang-2) and placental growth factor (PlGF) along with the appearance of narrow capillaries, and a decrease of blood vessel size. Ang-2, which supports the formation of mature blood vessels, furthermore was proven to be down-regulated by JWH-133 in gliomas and astrocytomas (Blázquez et al., 2003, 2004).

Among proteolytic enzymes involved in angiogenesis, the proangiogenic factor MMP-2 was demonstrated to be down-regulated by THC in human tumor samples from recurrent glioblastoma multiforme as well as in nude mice xenografted with the subclone from rat glioma C6 cells, C6.9 (Blázquez et al., 2008b). By contrast, THC left MMP-2 expression in the nonresponder subclone C6.4 virtually unaltered (Blázquez et al., 2008b). MMP-2 expression is also diminished in vitro in cervical cancer cells by THC and methanandamide (MA) (Ramer and Hinz, 2008) and in vivo in glioma xenografts treated with JWH-133 (Blázquez et al., 2003) accompanied by reduced invasiveness of cancer cells and impaired tumor vasculature, respectively. Finally, Pisanti et al. (2007) were able to demonstrate an inhibition of MMP-2 activity in endothelial cells incubated with Met-F-AEA.

There are also studies addressing the impact of cannabinoids on antiangiogenic factors. According to investigations by Casanova et al. (2003), the expression of thrombospondin-1 and -2, both multidomain matrix glycoproteins with inhibitory action on neovascularization, was not influenced upon treatment

with WIN-55,212-2 and JWH-133 in nude mice xenografted with melanoma carcinoma cells.

The expression pattern of the tissue inhibitor of metalloproteinases-1 (TIMP-1), which acts as an inhibitor of angiogenesis (Seandel et al., 2001), has been controversial in experiments assessing the influence of cannabinoids on this mediator. On the one hand, cannabinoids up-regulate TIMP-1 expression in human cervical and lung cancer cells as part of a mechanism contributing to its anti-invasive action (Ramer and Hinz, 2008). On the other hand, the same inhibitor was down-regulated upon treatment with THC in different glioma cell lines as well as in human tumor samples from recurrent glioblastoma multiforme patients (Blázquez et al., 2008a). In the latter study, a TIMP-1-lowering effect was likewise elicited by the selective CB_2 agonist JWH-133 in nude mice xenografted with C6.9 glioma cells.

Interestingly, HU-331, a cannabinoid quinone derived from the poor agonist for cannabinoid receptors, cannabidiol (CBD), seems to exert its antiangiogenic action via mechanisms that profoundly differ from those demonstrated for several other cannabinoids. According to Kogan et al. (2006), HU-331 inhibits angiogenesis by directly inducing apoptosis of vascular endothelial cells without changing the expression of pro- and antiangiogenic factors and their receptors. In a subsequent study, HU-331 has been reported to mediate its antitumorigenic action mainly via inhibition of topoisomerase II (Kogan et al., 2007).

Collectively, cannabinoids may act antiangiogenic by disposing tumor cells to decrease the production of proangiogenic factors and/or by direct modulation of endothelial cells. Therefore, cannabinoid receptor agonists as well as cannabinoid quinones with topoisomerase II inhibitory activity may provide a promising tool for antiangiogenic strategies in cancer treatment. An overview concerning the findings published in this field is given in Table 2.

Effects of Cannabinoids on Tumor Cell Migration. Besides the involvement in physiological processes, such as embryogenesis, wound healing, and immune responses, cellular migration represents an important step in tumor spreading (for review, see Lauffenburger and Horwitz, 1996). In particular, cell migration is crucial for the spread of cancer once a tumor reaches a specific size and becomes metastatic. To spread into tissues of distant organs, the primary tumor has to enter lymphatic or blood vessels.

TABLE 2
Overview on proangiogenic factors investigated for modulation by cannabinoids

Angiogenic Factor	Cannabinoid	Tumor Type	Regulation		References
			In Vitro	In Vivo	
Ang-2	JWH-133	Glioma, skin, astrocytoma	–	↓	Blázquez et al., 2003, 2004; Casanova et al., 2003
	WIN-55,212-2	Skin	–	↓	Casanova et al., 2003
EGFR	JWH-133	Skin	↓	↓	Casanova et al., 2003
	WIN-55,212-2	Skin	↓	↓	Casanova et al., 2003
	HU-210	Glioblastoma	↔	–	Galve-Roperh et al., 2002
	WIN-55,212-2 THC, HU-210, AEA	Squamous cell carcinoma, lung, bladder	↑	–	Hart et al., 2004
	THC, AEA	Astrocytoma, kidney cancer	↑	–	Hart et al., 2004
	THC	Glioma	↑	–	Hart et al., 2004
	THC	Lung	↔	–	Preet et al., 2008
Heme oxygenase-1	JWH-133	Glioma	–	↓	Blázquez et al., 2004
HIF-1α	JWH-133	Glioma	–	↓	Blázquez et al., 2004
Id3	JWH-133	Glioma	–	↓	Blázquez et al., 2004
Midkine	JWH-133	Glioma	–	↓	Blázquez et al., 2004
MMP-2	JWH-133	Glioma, Astrocytoma	–	↓	Blázquez et al., 2003
	JWH-133	Glioma	–	↓	Blázquez et al., 2008b
	THC	Glioma	↓	↓	Blázquez et al., 2008b
		Cervical	↓	–	Ramer and Hinz, 2008
	MA	Cervical	↓	–	Ramer and Hinz, 2008
MMP-3	THC, JWH-133	Glioma	–	↔	Blázquez et al., 2008b
MMP-9	THC, JWH-133	Glioma	–	↔	Blázquez et al., 2008b
	THC, MA	Cervical	↔	–	Ramer and Hinz, 2008
MT1-MMP	THC, JWH-133	Glioma	–	↔	Blázquez et al., 2008b
PIGF	JWH-133	Skin	–	↓	Casanova et al., 2003
	WIN-55,212-2	Skin	–	↓	Casanova et al., 2003
Tie-1	JWH-133	Glioma	–	↓	Blázquez et al., 2004
VEGF	JWH-133	Glioma, Skin	–	↓	Blázquez et al., 2003; Casanova et al., 2003
	JWH-133	Glioma	↓	↓	Blázquez et al., 2004
	WIN-55,212-2	Skin	–	↓	Casanova et al., 2003
	WIN-55,212-2	Glioma, Skin, Astrocytoma, Bladder	↓	–	Blázquez et al., 2004
	AEA	Glioma	↓	–	Blázquez et al., 2004
	Met-F-AEA	Thyroid	↓	↓	Portella et al., 2003
	THC	Lung	↓	–	Preet et al., 2008
VEGFR-1	Met-F-AEA	Thyroid	↓	↓	Portella et al., 2003
VEGFR-2	JWH-133	Glioma	↔	↔	Blázquez et al., 2004
	WIN-55,212-2	Glioma	↔	–	Blázquez et al., 2004
	THC	Glioma	–	↓	Blázquez et al., 2004
VEGFR-2 (activation)	JWH-133	Glioma	↓	↓	Blázquez et al., 2004
	WIN-55,212-2	Glioma	↓	–	Blázquez et al., 2004
	THC	Glioma	–	↓	Blázquez et al., 2004

↑, up-regulated/activated; ↓, down-regulated/deactivated; ↔, not influenced; –, not determined.

Migration of cancer cells is initiated by paracrine or endocrine chemoattractants. Among the chemoattractants that trigger migration, cell growth, proliferation, and differentiation, the EGF and its cognate receptor, EGFR, are considered to play a pivotal role. According to Preet et al. (2008), THC elicits a decrease of EGF-induced migration of NSCLC cells as assessed by scratch wound and Transwell migration experiments but leaves basal migration virtually unaltered. In this study, intracellular signaling events downstream to EGFR, such as inhibition of mitogen-activated protein kinases and protein kinase B (Akt) activity, were detected as targets of cannabinoid action rather than a direct inhibition of EGFR activation (Preet et al., 2008). Conflicting data have been published regarding the impact of cannabinoids on EGFR activation. In one study, cannabinoid receptor agonists have been shown to induce glioma and lung carcinoma cell proliferation via cannabinoid-induced EGFR signal transactivation (Hart et al., 2004). In contrast to these findings, other studies revealed inhibitory actions of WIN-55,212-2 and JWH-133 on EGFR activation in skin tumors in vivo (Casanova et al., 2003) and of AEA on EGFR expression and EGFR-induced proliferation of prostate cancer cells with the latter effect occurring in a CB_1-dependent manner (Mimeault et al., 2003). Finally, one investigation reported no alteration of EGFR tyrosine phosphorylation by cannabinoids in human astrocytoma cells (Galve-Roperh et al., 2002).

Other studies ascribe neurotransmitters a role in regulating cell migration (Entschladen et al., 1997). In this context, Joseph et al. (2004) demonstrated an inhibitory action of different cannabinoids on norepinephrine-induced cancer cell migration. Whereas AEA, the synthetic cannabinoid HU-210, and the AEA analog docosatetraenylethanolamide (DEA) blocked migration of colon carcinoma cells with low CB_2 receptor expression, JWH-133 had no influence in this respect. These findings suggest a pivotal role of the CB_1 receptor in the antimigratory action given that AEA and HU-210 activate both cannabinoid receptors, DEA acts as a CB_1 receptor agonist, and JWH-133 triggers an intrinsic activity on CB_2 receptors only (Joseph et al., 2004). It is noteworthy that the concept of an antimigrative effect on tumor cells that involves CB_1 rather than CB_2 receptor signaling, thereby sparing unwanted side effects of cannabinoids on the recruitment of immune cells as suggested by the authors, contradicts findings that even favor a CB_2-mediated antitumorigenic action that spares psychoactive side effects (Blázquez et al., 2003).

In a more recent study, Grimaldi et al. (2006) reported a CB_1 receptor-dependent antimigrative effect for Met-F-AEA on breast cancer cells. Laezza et al. (2008), who confirmed this finding, demonstrated an involvement of the RhoA/Rho-associated coiled coil-containing kinase (RHOA-ROCK) system in the antimigratory action of this cannabinoid. Accordingly, Met-F-AEA inhibits the activity of the GTPase RHOA and causes a RHOA delocalization from the cell membrane to the cytosol, which in turn results in alterations in the actin cytoskeleton (Laezza et al., 2008).

Numerous findings support a relationship between mast cell activation, enhanced tumor growth, and tumor progression (for review, see Cheng et al., 2006). Accordingly, mast cells were recently demonstrated as a source of promigrative chemoattractants acting as possible targets of cannabinoids (Rudolph et al., 2008). In the latter study, cancer cell migration initiated by mast cells was down-regulated by the endocannabinoid 2-arachidonylglycerol (2-AG) as well as by the synthetic cannabinoid WIN-55,212-2 in the scratch wound healing assay. In both cases, this down-regulation was CB_1 receptor-dependent.

Furthermore, a receptor-independent inhibition of human glioma cell migration was demonstrated for the weak psychoactive cannabinoid CBD (Vaccani et al., 2005). In this study, neither cannabinoid receptor antagonists nor pertussis toxin were able to reverse the antimigratory action of CBD, excluding the involvement of $G_{i/o}$ protein-coupled receptor signaling in general. Finally, in our hands, MA and THC left the basal migration of human cervical and lung cancer cells virtually unaltered (Ramer and Hinz, 2008), implicating a cell type-specific and/or chemoattractant-dependent regulation of migration by cannabinoids. In summary, the currently available data (for summary, see Table 3) suggest an antimigratory potency of cannabinoids, with the underlying signal pathways still requiring further investigation.

Influence of Cannabinoids on the Adhesion of Cancer Cells. Adhesive interaction of tumor cells with the surrounding microenvironment (e.g., tumor-stroma interaction, attachment of endothelial cells to tumor tissue) represents a crucial parameter within growth, migration and metastasization of cancer cells. Adhesion to extracellular matrix (ECM) is conferred by matrix proteins, such as integrins, cadherins, selectins, and cell adhesion molecules of the immunoglobulin superfamily (IgSF CAMs).

Findings concerning the influence of cannabinoids on the adhesion of cancer cells are still rare. In this context, Grimaldi et al. (2006) have shown that the AEA analog Met-F-AEA selectively reduced the adhesion of human breast cancer cells to the ECM component collagen type IV in vitro but had no effect on the adhesion to fibronectin and laminin. As the underlying mechanism, a CB_1 receptor-dependent signal transduction pathway was identified. It is noteworthy that Met-F-AEA did not affect integrins at the level of expression but decreased their affinity to collagen by suppressing phosphorylation of the focal adhesion kinase (FAK) and the proto-oncogenic tyrosine kinase Src. Controversial findings were obtained by Preet et al. (2008) using human NSCLC cells. In these cells, THC was shown to enhance the phosphorylation of FAK in vitro but decrease its phosphorylation in vivo. In both experiments, the expression of total FAK protein was unaffected (Preet et al., 2008). Finally, in experiments published by Zhou and Song (2002), the synthetic cannabinoid HU-210 was devoid of a direct influence on FAK phosphorylation in murine neuroblastoma cells (Zhou and Song, 2002). In these cells, another factor, the FAK-related nonkinase (FRNK), that is supposed to regulate the activity of FAK as an inhibitor (Richardson and Parsons, 1996; Gervais et al., 1998; Sieg et al., 1999), was phosphorylated in a CB_1 receptor-dependent manner (Zhou and Song, 2002).

In another study, Curran et al. (2005) observed that the intercellular cell adhesion molecule 1 and the vascular cell adhesion molecule 1, which belong to the IgSF CAMs, are also influenced by cannabinoids. In their hands, the synthetic cannabinoid WIN-55,212-2 blocked the interleukin 1 (IL-1)-induced up-regulation of intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1 in human glioblastoma and lymphoma cells in a cannabinoid receptor-independent manner. As the underlying mechanism, WIN-

TABLE 3
Overview on the functional effects of cannabinoids on tumor cell migration, adhesion, invasion, and metastasization

Cannabinoid	Tumor Type	Regulation of Functional Effect	Signal Transduction	References
Migration				
AEA	Colon	↓	CB_1 receptor	Joseph et al., 2004
	Breast	↓	CB_1 receptor	Joseph et al., 2004
2-AG	Cervical	↓	CB_1 receptor	Rudolph et al., 2008
CBD	Glioma	↓	Independent of $G_{i/o}$-protein-coupled receptors	Vaccani et al., 2005
DEA	Colon	↓	CB_1 receptor	Joseph et al., 2004
HU-210	Colon	↓	CB_1 receptor	Joseph et al., 2004
JWH-133	Colon	↔	–	Joseph et al., 2004
MA	Cervical, Lung	↔	–	Ramer and Hinz, 2008
Met-F-AEA	Breast	↓	CB_1 receptor	Grimaldi et al., 2006
		↓	RHOA-ROCK-dependent	Laezza et al., 2008
THC	Lung	↓	Inhibition of EGF-induced ERK1/2, JNK, AKT; increased phosphorylation of FAK	Preet et al., 2008
	Glioma, Astrocytoma	↓	–	Blázquez et al., 2008a
	Cervical, Lung	↔	–	Ramer and Hinz, 2008
WIN-55,212-2	Cervical	↓	CB_1 receptor	Rudolph et al., 2008
Adhesion/adhesive proteins				
HU-210	Neuroblastoma	-	CB_1 receptor; up-regulation of FRNK phosphorylation	Zhou et al., 2002
Met-F-AEA	Breast	↓	CB_1 receptor; inhibition of FAK-, and Src-phosphorylation on collagen type IV	Grimaldi et al., 2006
WIN-55,212-2	Glioma	-	Blockade of IL-1-induced up-regulation of cell adhesion molecules	Curran et al., 2005
	Astrocytoma	-	Cannabinoid receptor-independent blockade of IL-1-induced up-regulation of cell adhesion molecules	Curran et al., 2005
Invasion				
2-AG	Prostate	↓	CB_1	Nithipatakom et al., 2004
CBD	Breast	↓	Id-1 down-regulation	McAllister et al., 2007
MA	Cervical	↓	CB_1, CB_2 receptors, TRPV1; TIMP-1 up-regulation	Ramer and Hinz, 2008
	Lung	↓	CB_1, CB_2 receptors, TRPV1; TIMP-1 up-regulation	Ramer and Hinz, 2008
THC	Cervical	↓	CB_1, CB_2 receptors; TIMP-1 up-regulation	Ramer and Hinz, 2008
	Lung	↓	CB_1, CB_2 receptors; TIMP-1 up-regulation	Ramer and Hinz, 2008
	Lung	↓	Inhibition of EGF-induced ERK1/2, JNK, AKT; increased phosphorylation of FAK	Preet et al., 2008
	Glioma	↓	MMP-2 down-regulation	Blázquez et al., 2008b
Metastasization				
CBD-rich extract	Breast	↓	Ca^{2+}, ROS	Ligresti et al., 2006
CBD	Breast	↓	Ca^{2+}, ROS	Ligresti et al., 2006
Met-F-AEA	Breast	↓	CB_1 receptor	Grimaldi et al., 2006
	Lung	↓	CB_1 receptor	Portella et al., 2003
THC	Lung	↓	Inhibition of FAK-, ERK1/2- and Akt-phosphorylation,	Preet et al., 2008
	Breast	↑	–	McKallip et al., 2005
WIN-55,212-2	Skin	↓	Inhibition of the Akt signal transduction pathway	Blázquez et al., 2006

↑, Up-regulated; ↓, down-regulated; ↔, not influenced; –, not determined.

55,212-2 was shown to inhibit IL-1-induced activation of the transcription factor nuclear factor κB, a key regulator in the expression of cell adhesion molecules (Curran et al., 2005). In conclusion, the initial but limited data (for summary, see Table 3) imply that cannabinoids may decrease the adhesion of cancer cells to the adjacent microenvironment, thereby exerting a beneficial impact on tumor development.

Effects of Cannabinoids on Tumor Cell Invasion. Cancer cell invasion is one of the crucial events in local spreading, growth, and metastasis of tumors. First evidence suggesting an anti-invasive action was published by Nithipatikom et al. (2004) who showed that 2-AG inhibits invasion of androgen-independent prostate cancer cells by a mechanism involving CB_1 receptor activation. However, the precise mechanism leading to decreased invasiveness by cannabinoids remained elusive. Recently, several investigations have provided new insight into how cannabinoids could achieve their anti-invasive action.

In this context, several studies suggest a modulation of the MMP system by cannabinoids as part of their anti-invasive action. MMPs belong to a group of enzymes exerting an important function during tumor invasion, metastasis, and angiogenesis through degradation of ECM components (Curran and Murray, 2000; Stamenkovic, 2000). Of all MMPs, particularly MMP-2 and -9, are known to facilitate tumor invasion by proteolytic degradation of major basement membrane components, such as type IV collagen, laminin, and nidogen (for review, see Curran and Murray, 2000). The activity of MMPs is attenuated by specific TIMPs that bind noncovalently in a 1:1 stoichiometric fashion to the active forms of MMPs, thereby inhibiting the proteolytic activity of these enzymes. Consequently, an imbalance between MMPs and TIMPs toward increased proteolytic activities is associated with higher ECM degradation necessary for tumor cell invasion and metastasis.

First evidence for a direct effect of cannabinoids on the

MMP system was published by Blázquez et al. (2003) who observed a JHW-133-mediated decreased expression and activity of MMP-2 in mice xenografted with a rat glioma cell line and human grade IV astrocytoma cells obtained from tumor biopsies. More recently, Pisanti et al. (2007) demonstrated inhibition of MMP-2 activity by Met-F-AEA that confers an antiangiogenic action. Using cultured glioma cells, Blázquez et al. (2008b) reported inhibition of MMP-2 expression and cell invasion by THC. In the latter study, modulation of MMP-2 expression by RNA interference and cDNA overexpression experiments proved that down-regulation of this MMP plays a critical role in THC-mediated inhibition of cell invasion. Moreover, cannabinoid-induced inhibition of MMP-2 expression and cell invasion was prevented by blocking ceramide biosynthesis and by knocking down the expression of the stress protein p8 (Blázquez et al., 2008b). Using cervical carcinoma cells, we observed a concentration-dependent inhibition of MMP-2 expression by MA and THC that was, however, independent of cannabinoid receptor and TRPV1 activation (Ramer and Hinz, 2008). In the cell line tested (HeLa), the cannabinoid-mediated decrease of MMP-2 expression was not considered to be of significance in the anti-invasive action of cannabinoids given that the basal invasion of HeLa depends on MMP-9 rather than on MMP-2 as assessed by small interference RNA approaches (Ramer and Hinz, 2008).

Other findings imply an involvement of TIMP-1 in the anti-invasive effects of cannabinoids. Among the four distinct members of the TIMP family, the 28.5-kDa glycoprotein TIMP-1 has emerged as a potent MMP inhibitor that suppresses vascular tumor growth, angiogenesis, and cancer-induced osteolysis in tumor-bearing animals (Zacchigna et al., 2004; Deng et al., 2008). In addition, several studies demonstrated a correlation between high cancer invasiveness and decreased TIMP-1 expression (Khokha et al., 1989; Chan et al., 2005). Likewise, the anti-invasive action of several anticarcinogenic drugs has been associated with elevated TIMP-1 levels (Khokha et al., 1992; Cattaneo et al., 2005; Park et al., 2005a,b; Ramer et al., 2007). On the other hand, TIMP-1 up-regulation has also been reported to be associated with poor patient prognosis (Hornebeck et al., 2005). It has been demonstrated in this context that TIMP-1 may also possess MMP-independent antiapoptotic properties (Hornebeck et al., 2005), suggesting a distinct influence of this molecule in tumor progression depending on cancer cell type. In line with the potential anti-invasive action of TIMP-1, we have recently shown that the anti-invasive action of MA and THC strongly depends on the induction of TIMP-1 expression in cervical carcinoma and lung cancer cells (Ramer and Hinz, 2008). In our hands, the decrease of invasiveness by THC was even significant at concentrations as low as 0.01 μM (68% inhibition). With reference to the fact that in humans average peak plasma concentrations of 0.03 and 0.045 μM can be obtained after oral doses of 15 and 20 mg of THC (Wall et al., 1983), the effects of THC on cell invasion were observed at therapeutically relevant concentrations. The expression of TIMP-1 was shown to be stimulated by CB_1/CB_2 receptor activation and, in the case of MA, by additional activation of TRPV1. Further experiments addressing the signaling events underlying increased TIMP-1 expression revealed a contribution of the p38 mitogen-activated protein kinase and the extracellular regulating kinases 1 and 2 (ERK 1/2) to this process. In contrast to these findings, Blázquez et al. (2008a) reported a cannabinoid-induced inhibition of TIMP-1 expression in various glioma cell lines as well as in primary tumor cells obtained from glioblastoma multiforme tissues. As previously reported for cannabinoid-induced apoptosis, this effect was dependent on de novo synthesis of ceramide. Thus, cannabinoid action on TIMP-1 expression and the subsequent impact on tumorigenesis of the latter may depend on tumor type.

It is interesting that the inhibition EGF-induced Matrigel invasion by THC in NSCLC cells (Preet et al., 2008) seems to involve a mechanism that differs from the pathways of fetal calf serum-induced invasion described above (Ramer and Hinz, 2008). Accordingly, Preet et al. (2008) demonstrated an anti-invasive effect of THC on EGF-induced invasion that was accompanied by reduced transwell migration and migration monitored by scratch wound healing. In our hands, serum-induced Matrigel invasion assessed with the same cell line (A549) revealed a selective inhibition of Matrigel invasion by THC without altering the migration through uncoated transwell inserts, suggesting modulation of the proteolytic impact on surrounding matrix components as a crucial parameter of THC-mediated inhibition of cancer cell invasion (Ramer and Hinz, 2008). Due to the fact that in both studies toxic effect of THC on tumor cells were excluded, it is tempting to speculate that the mechanisms of cannabinoid action on tumor cell invasion is furthermore dependent on the respective chemoattractant.

With regard to the poor cannabinoid receptor agonist CBD, McAllister et al. (2007) reported that this nonpsychoactive compound may down-regulate the expression of the DNA-binding protein inhibitor 1 (Id-1) in aggressive human breast cancer cells. Id-1 is an inhibitor of basic helix-loop-helix transcription factors that represents a key regulator of the metastatic potential of breast and additional cancers (Fong et al., 2003). Evidence for a role of Id-1 in the anti-invasive action of CBD was provided by experiments demonstrating that ectopic expression of Id-1 in breast cancer cells abolished the effects of CBD on cell invasion (McAllister et al., 2007).

Collectively, the contemporary available data (for summary, see Table 3) suggest an anti-invasive effect of cannabinoids mediated by down-regulation and/or inhibition of matrix degrading enzymes. Due to the complex tumor-stroma interaction, more research is needed to further define the influence of cannabinoids on other matrix interactions that modulate tumor invasion.

Effects of Cannabinoids on Tumor Cell Metastasization. Metastasization represents the transfer of a malignant tumor from one area to a distant organ. Although only 1% of micrometastases expands into macrometastases, metastasization is the most frequent reason for death of cancer patients. In the previous studies, cannabinoids were reported to reduce adhesion, angiogenesis, migration, and invasion by several ways. Because these processes are parts of the progression of metastases, cannabinoids are expected to influence the development of metastases in a similar way.

Accordingly, experiments using breast cancer cell lines obtained a reduction of lung metastatic nodes by Met-F-AEA via a CB_1 receptor-dependent pathway (Grimaldi et al., 2006). In line with these observations, Portella et al. (2003) demonstrated a CB_1 receptor-dependent reduction of murine Lewis lung carcinoma metastasization. In their experiments, Met-F-AEA dramatically inhibited metastasization, and the few metastases that were found were smaller in size. In the

same study, the authors additionally reported an impaired proliferation of metastasis-derived rat thyroid and lung cancer cell lines by Met-F-AEA.

In contrast, McKallip et al. (2005) demonstrated a THC-elicited increased number of lung metastases after injection of murine mammary cell carcinoma cells to mice with the dimension of lung nodules enlarging proportionally to the administered dose of THC. It is noteworthy that these experiments were carried out with murine cells expressing low levels of CB_1/CB_2 receptors, giving one possible explanation for the apparent difference to findings obtained with human cells. Another potential reason discussed by the authors involves the suppression of the antitumor immune response by THC. However, further experiments in mice injected with lung cancer cells showed a reduction of surface lung metastases through THC (Preet et al., 2008).

The signal transduction pathway involved in the antimetastatic cannabinoid action is not fully clarified, but it is obvious that FAK, ERK1/2, and Akt are involved in this process. Accordingly, Preet et al. (2008) detected a dephosphorylation of ERK1/2 and Akt and an increased phosphorylation of FAK following treatment of lung cancer cells with THC. In line with these findings, Blázquez et al. (2006) suggested an involvement of Akt dephosphorylation in the antimetastatic action of WIN-55,212-2 on melanoma cells in vivo.

In breast cancer cells, Id-1, mentioned in context with invasion earlier in this review, was shown to be downregulated by CBD (McAllister et al., 2007). As animal experiments revealed that a reduction of Id-1 is associated with decreased breast cancer metastases (Fong et al., 2003; Minn et al., 2005), CBD is expected to exert antimetastatic properties. In fact, Ligresti et al. (2006) from the group of Vincenzo Di Marzo were able to demonstrate an inhibitory action of a CBD-rich extract and CBD on the metastatic potential of breast cancer cells in vivo resulting in a decrease of metastatic lung nodules far under half of those counted in control animals.

Collectively, cannabinoids seem to reduce the expansion of tumor cells by several signal transduction pathways. An overview on the effects of different cannabinoids on metastasization is given in Table 3.

Conclusion

Recent investigations have shown that besides its well known antiapoptotic and antiproliferative action, cannabinoids may also confer antiangiogenic, antimigrative, antiadhesive, anti-invasive, and antimetastatic properties by pathways including activation of both cannabinoid receptors as well as TRPV1. Although a limited number of studies have been published addressing the underlying mechanisms, the currently available results indicate that the modulation of several components of signal transduction pathways, including Src, nuclear factor κB, ERK1/2, HIF-1α, Akt, and modulation of the expression as well as that of the enzymatic action of proteins of the MMP family, EGF, VEGF, IgSF CAMs, and FAK, by cannabinoids might support beneficial effects on tumor cell locomotion and spreading. Based on these facts, evidence is emerging to suggest that cannabinoids are potent inhibitors of both cancer growth and spreading. Because cannabinoids are usually well tolerated and do not develop the toxic effects of conventional chemotherapeutics, more preclinical studies are warranted to investigate a potential utility of these substances as anticancer therapeutics.

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Address correspondence to: Dr. Burkhard Hinz, Institute of Toxicology and Pharmacology, University of Rostock, Schillingallee 70, 18057 Rostock, Germany. E-mail: burkhard.hinz@med.uni-rostock.de