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Visar o sistema endocanabinóide para tratar melanoma cutâneo: uma revisão sistemática

Targeting the endocannabinoid system to treat skin melanoma: a systematic review

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TÍTULO DISSERTAÇÃO

Targeting the endocannabinoid system to treat skin melanoma: a systematic review

ORIENTADOR

Doutora Inês Bastos Correia de Sá

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É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☒
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Faculdade de Medicina da Universidade do Porto, 03/03/2020

Assinatura conforme cartão de identificação: Nuno Falcão

Aos que nunca me deixaram cair

E à minha querida irmã, na saúde e na doença

Targeting the endocannabinoid system to treat skin melanoma: a systematic review.

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Abstract

Introduction: Skin melanoma is a malignant neoplasm of melanocytes and is the deadliest of all skin cancers. It is a highly metastatic neoplasia, for which conventional therapy is often ineffective. The endocannabinoid system is implicated in proliferation, differentiation and survival of skin melanocytes. In this study, we collect published data regarding the role of cannabinoids, cannabinoid 1 (CB1) and cannabinoid 2 (CB2) receptors in the treatment of skin melanoma.

Methods: PRISMA guidelines were followed for a systematic literature review of published data accessing the role of cannabinoids on melanoma treatment. A PUBMED search was conducted, for published studies until January 2020, using the queries: "Cannabinoid AND Melanoma", "CB1 AND Melanoma" and "CB2 AND Melanoma".

Results: Of the 116 articles retrieved, 11 were included in this review.

Conclusion: CB1 and CB2 activation is responsible for inhibiting melanoma cell growth *in vitro*, mostly by cell-cycle arrest and induction of apoptosis. Membrane lipid rafts and GPR55 are also involved in cannabinoid action. Cannabinoids decrease tumor growth and metastasis *in vivo*. The endocannabinoid system may be a relevant target of melanoma treatment.

Key words: Endocannabinoid system, cannabinoid, melanoma, CB1, CB2.

Introduction

The Endocannabinoid System in the Skin

The Endocannabinoid System (ECS) in any tissue is composed of several elements. It includes endocannabinoids, which are cannabinoids produced endogenously on demand from membrane lipids. These are structurally related to Δ^9-tetrahydrocannabinol (THC, the main active ingredient of the *Cannabis sativa* plant) and exert similar effects to THC [1, 2]. The most studied endocannabinoids are anandamide (N-arachidonoylethanolamide; AEA) and 2-arachidonoylglycerol (2-AG). The ECS also includes the enzymes required to synthetize, transport and degrade endocannabinoids. AEA synthesis is carried out by the enzyme phospholipase D, and 2-AG by diacylglycerol lipase (DAGL). AEA is mainly degraded by fatty acid amide hydrolase (FAAH), and 2-AG by monoacylglycerol lipase (MAGL) [1, 2]. Finally, the ECS includes both CB1 and CB2 receptors, which are the two main G protein-coupled membrane cannabinoid receptors (GPCRs) responsible for the cellular effects of cannabinoids [1]. CB1 and CB2 receptors are coupled to $G_{i/o}$ proteins [3].

A fully functional ECS comprised of the above elements has already been described in skin tissue [1, 2, 4]; namely in skin fibroblasts, keratinocytes and melanocytes [2]. CB1 and CB2 receptors have also been found in cells of dermal appendages, specifically in hair follicles and sebaceous glands [2, 5]. CB1 and CB2 expression has also been documented in cells of the skins' immune system, in resident mast cells and macrophages, as well as in T and B lymphocytes [2].

The ECS is involved in many biological functions. The expression of CB1 and CB2 receptors was first described in the central nervous system and in peripheral immune

cells respectively [1]. ECS has been involved in learning, memory, neuroprotection, immune response, among others [2]. The ECS has been implicated in well-balanced skin cell proliferation, differentiation, and survival; mainly by CB1 and CB2 activation [1, 2, 6]. Immune and inflammatory responses initiated in skin tissue are mostly mediated by the CB2 receptor [2]. Nevertheless, the physiological functions of the ECS in the skin are also mediated by a series of other membrane and nuclear receptors, such as the Transient Receptor Potential Vanilloid-1 Channel (TRPV1) and Peroxisome Proliferator Activated Receptor Gamma (PPARγ). The TRPV1 channel is a membrane channel that appears to be responsible, under physiological conditions, for the same functions above described for the CB1 and CB2 receptors [1, 2].

Melanoma and the Endocannabinoid System

Skin cancer can be divided in two subtypes, Melanoma and Non-Melanoma Skin Cancer (NMSC) [7]. Melanoma is a malignant neoplasia originated in melanocytes; which are found not only in the skin, but also in mucosae, meninges and the eye uvea [8]. More than 90% of cases of melanoma occur in the skin [9]. The vast majority of melanoma cases are sporadic and related to a single predisposing risk-factor: exposure to ultraviolet radiation (UVR), either UV-A or UV-B [8, 10]. UVR can arise from exposure to sunlight, tanning beds and sun lamps, although exposure to sunlight is the main source [10]. In addition, it appears that severe sunburns in early ages is the most important risk factor [8]. UVR induces DNA damage and can lead to oncogenes activation and tumor suppressor genes inactivation [11]. UV-B also stimulates cell proliferation, at least partly by activation of cell surface receptors for epidermal growth factor (EGF), TNF-α and IL-1 [11].

While NMSC is by far the most common skin cancer, standing as the 5th most common cancer worldwide in women and men [12]; melanoma is the deadliest of all skin cancers, albeit only being the 19th most common cancer worldwide [12, 13]. This difference can be explained by the fact that melanoma is a highly metastatic neoplasia, often resistant to conventional chemotherapy and radiation therapy, explaining the low survival rates once metastization occurs (mean survival of 6 to 9 months) [7, 13-15]. According to treatment guidelines from the NCCN (Nacional Comprehensive Cancer Network), the basis of melanoma treatment is surgery. Wide excision technique is the gold-standard, even for stage 0 tumors (*in situ* melanomas). For unresectable metastatic tumors (stage IV), there are several immunotherapies and targeted therapies (for BRAF mutated melanomas) available, but prognosis remains poor [16]. Melanoma still remains a surgical disease, since the best chance of cure is still the removal of thin, biologically early tumors [17].

The ECS in healthy skin and melanocytes plays an important role in proliferation, differentiation and survival; but its role in melanoma is slightly more controversial. *In vitro* studies using human melanoma cell lines suggest that the ECS is involved in the pathogenesis of melanoma [3, 11, 18]. On the other hand, *in vivo* studies with mice suggest that both CB1 and CB2 receptors are not involved in the pathogenesis of chemically induced melanomas [19].

Despite the conflicting data, research has been focusing on the therapeutic use of cannabinoid-based drugs or ECS-modulating drugs, in the treatment of melanoma and other cancers [20]. The use of cannabinoid-based drugs in the palliative treatment of cancer has already been established. These compounds have been approved for palliative therapy of cancer-associated symptoms as anorexia, weight lost and pain. They have also been used for chemotherapy-associated nausea and vomiting [21].

We therefore searched how targeting the ECS in melanoma can be useful as an alternative treatment in advanced melanoma.

Methods

This systematic review was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [22].

A systematic search in PUBMED for studies published in English until January 2020 on cannabinoids and melanoma was conducted. Articles in the PUBMED online database were searched using the queries: “Cannabinoid AND Melanoma”, “CB1 AND Melanoma”, “CB2 AND Melanoma”. Two independent authors identified the articles and extracted the data. Given the preliminary stage of research in this area, all manuscripts that directly pertain to the topic were included in this review. Only minimal exclusion criteria were used, and no restrictions on the design of the study were applied. We excluded reviews, letters, editorials and case reports. Of the original 116 articles, a total of 11 were included in this review.

Results

The initial data base search yielded 113 citations, from which 35 duplicate studies were excluded. When the bibliographies of relevant papers were screened, 3 further studies were considered relevant. Based on an initial screening of the titles and abstracts of the remaining 81 articles, 59 were excluded and 22 were selected for full text examination. Of these 22 publications, 9 did not address any of the outcomes of interest and 2 were reviews of the existing literature. Those 11 publications were excluded. Eleven publications met our eligibility criteria and were included in our review.

A PRISMA flow diagram **(Figure 1)** demonstrates the procedure followed to select the papers used in this systematic review. **Table 1** summarizes relevant data found in the included articles.

Discussion

In vitro Results

CB1 and CB2 targeting

Only three studies were found studying CB1 and CB2 receptors simultaneously, in cultured melanoma cells from human and murine origins [14, 17, 19]. Two of these found that stimulation of the CB1 and CB2 receptors reduces cell viability [14, 17]. THC and WIN-55,212–2 (CB1 and CB2 agonists) were able to reduce the number of viable cells *in vitro* [14, 17]. THC and WIN-55,212–2 action was selective for melanoma cells [14, 17].

Blazquez, Carracedo [17] showed that THC and WIN-55,212–2 inhibit cell-cycle progression in tumor cells by inhibiting the prosurvival protein Akt. Inhibition of Akt protein decreases phosphorylation of the pRb retinoblastoma protein [17], a regulator of the G1-S transition in cell-cycle. This increased the number of cells in the G1 phase and reduced the number of cells in the S phase [17]. Armstrong, Hill [14] later confirmed that THC exerts its effect by inhibiting the Akt protein. These studies found that, at higher concentrations, both agonists were able to induce apoptosis in tumorigenic cells [14, 17].

Armstrong, Hill [14] further enlightened the cellular mechanism of action of THC. THC induces apoptosis in melanoma cells by the activation of non-canonical autophagy. In response to endoplasmic reticulum stress, Tribbles homolog 3 (TRIB3) protein

inhibited the Akt protein and the mammalian target of rapamycin complex 1 (mTORC1) [14]. The same author found that THC activates autophagy in melanoma cells through the autophagy-related 7 (Atg7) protein, which activates the caspase 3 protein, an effector of the proapoptotic pathway. The knockdown of TRIB3 and Atg7 by small interfering RNA abrogated the apoptotic effects of THC [14]. Nevertheless, the molecular mechanisms connecting autophagy to cell death remains poorly understood [23]. Armstrong, Hill [14] also found that THC effects were enhanced when combined with cannabidiol (CBD), a non-psychoactive cannabinoid [14]. CBD is believed to exert its proapoptotic effect through the production of reactive oxygen species [24]. This indicates that the proapoptotic effect is enhanced due to different mechanisms of action [14].

Despite these findings, Glodde, Jakobs [19] couldn't replicate the results using THC to inhibit cell growth [19]. The author suggested that the lack of results was due to a poor expression level of the two receptors. This highlights the important role of both cannabinoid receptors in the effectiveness of THC activity [19].

CB1 targeting

Kenessey, Banki [25] targeted the CB1 receptor after identification of the cannabinoid receptor 1 gene (CNR1) and its mRNA [25]. Selective CB1 activation was accomplished by the use of the agonist anandamide (AEA), and the selective CB1 agonists Arachidonyl-2-chloroethylamide (ACEA) and 2-methyl-2-fluoro-anandamide (Met-F-AEA). CB1 inactivation was achieved by using the selective antagonist N-piperidinyl-iodophenyl-dichlorophenyl-methylpyrazole-carboxamide (AM251). All of these CB1 modulators induced apoptosis in HT168-M1, HT199 and WM35 human melanoma cell lines. The most potent effect was seen with the use of AM251. At higher concentrations, all CB1 modulators induced cell necrosis, parallel to apoptosis. AM251

was also able to arrest cell-cycle at the G2/M transition in the WM983B lineage. CB1 stimulation with AEA also had an antimigratory effect, demonstrated by a migration assay. This may contribute to an antimetastatic effect in vivo [25]. Notwithstanding, Kenessey, Banki [25] doesn't disclaim if the examined melanoma cell lines express the CB2 receptor. Since AEA is referred in literature as a CB1 and CB2 agonist [26], this may be a confounding factor.

Carpi, Fogli [27] later confirmed the AM251 antiproliferative effects in melanoma cells [27]. Here, AM251 reduced melanoma cell viability in a time and concentration-dependent manner. The involved mechanism was, again, cell-cycle arrest at the G2/M transition [27]. AM251 was also able to induce apoptosis. This was achieved by decreasing the expression of antiapoptotic BCL2 and Survivin protein expression and increasing the expression of the BAX proapoptotic gene. The effects of AM251 were GPR55 independent. AM251 action was selective for cancer cells in the implemented concentrations [27]. Carpi, Fogli [27] also reported that celecoxib (a COX-2 selective inhibitor) was equally able to induce cell toxicity. Celecoxib effect was independent of COX-2 inhibition, and none of the other COX-2 selective and non-selective COX inhibitors were able to reduce cell viability, alone or in combination with AM251 [27]. It has been described that celecoxib effect may rely on apoptosis-induction and cell-cycle arrest at the G2/M transition [28, 29].The toxicity of celecoxib and AM251 was amplified when both were used simultaneously, illustrating a possible synergic effect.

Adinolfi, Romanini [30] confirmed AEA proapoptotic effect found by Kenessey, Banki [25]. The authors found no traces of CB2 receptor expression, despite using the same melanoma cell line as Blazquez, Carracedo [17]. This was attributed to a clonal difference [30]. Through caspase 3 and caspase 7 activation, AEA induced apoptosis in a dose-dependent fashion. The effect was partially CB1 dependent. Co-incubation of AEA

with selective COX-2 and LOX inhibitors (two enzymes capable of AEA oxidation) reduced AEA cytotoxicity. This suggest that COX-2 and LOX AEA metabolites contribute to the effects of AEA. COX-2 metabolites of AEA have been suggested to be prostaglandin E2-ethanolamides, which have cytotoxic activity. Nevertheless, the precise biological role of these metabolites needs to be further elucidated [30, 31].

Hamtiaux, Masquelier [32] found that inhibiting FAAH, the main enzyme degrading AEA [1, 2], decreases cell viability. Cell toxicity was achieved by using the selective FAAH inhibitor URB597 and other two dual FAAH/MAGL inhibitors (MAFP and CAY10499). AEA and 2-AG also decreased tumoral cell viability, albeit without mentioning the mechanisms behind this effect [32]. Hamtiaux, Masquelier [32] found no expression of CB2 receptors in the cultured cells. Adinolfi, Romanini [30] later confirmed that co-incubation of AEA with URB597 potentiated the cytotoxicity of AEA. However, in this study, URB597 did not affect cell viability per se [30].

CB2 targeting

Only one study specifically targeted the CB2 receptor [33]. CB2 activation has been previously cited in literature to be able to improve barrier properties of the endothelial layer [34], downregulate adhesion molecules [35] and matrix metalloproteinases [36]. Hasko, Fazakas [33] showed that selective CB2 agonist JWH-133 was able to reduce the adhesion of human melanoma cells to human brain endothelial cells. This was accomplished when both lineages were pre-treated with JWH-133 and treated with the same compound during an adhesion assay. Pre-treatment of primary brain endothelial cells with JWH-133 also reduced the migration rate of melanoma cells, with a bigger reduction when both cell types were pre-treated with JWH-133 [33]. CB2 activation is therefore a potential target to prevent melanoma brain metastasis [33].

Other receptors/mechanisms

Hamtiaux, Masquelier [32] demonstrated that N-palmitoylethanolamine (PEA), an endogenous mediator associated to the ECS, exerts its effects without binding to the CB1 and CB2 receptors [32]. This cannabinoid was reported to act as an "entourage" agent, able to increase AEA antiproliferative effects [37, 38]. Hamtiaux, Masquelier [32] demonstrated that PEA has a cytotoxic effect on melanoma cells in a dose-dependent manner [32]. The mechanism behind this effect remains to be explained; since inhibition of the classical CB1, TRPV1, PPARα, PPARγ and GPR55 receptors did not reduce the effect of PEA. PEA is degraded in the cells by the FAAH enzyme. URB597 was able to increase PEA cellular levels, and co-incubation of PEA and URB597 increased cell apoptosis and necrosis [32].

It was previously mentioned that WIN-55,212–2 (a mixed CB1 and CB2 agonist) reduced cell viability in a CB dependent manner [14, 17]. Scuderi, Cantarella [39] suggests a different mechanism for the proapoptotic effect of WIN-55,212–2. The author claims that WIN-55,212–2 mediates cell death due to a mechanism involving membrane lipids rafts and activation of the intrinsic caspase pathway, leading to cell apoptosis. The lipid raft disruptor methyl-beta-cyclodestrin (MCD) was able to partially rescue melanoma cell lines from apoptotic death [39]. WIN-55,212–2 induced phosphorylation of the extracellular signal-regulated kinase (ERK) [39], which literature suggests has a proapoptotic action [40]. Inhibition of CB1 and CB2 receptors was not able to abolish WIN-55,212–2 cell death induction [39]. In Adinolfi, Romanini [30] study, MCD treatment was able to rescue AEA treated melanoma cells from death. This demonstrates the possible role of lipid rafts in cannabinoid action [30, 39].

Adinolfi, Romanini [30] found another mechanism for melanoma cell death. Direct stimulation of GPR55 (a G-protein-coupled receptor associated with the ECS) by

a selective agonist (O-1602) was also able to induce dose-dependent cell death [30]. MCD was also able to fully reverse O-1602-induced cytotoxicity [30]. Again, this indicates a role of lipid rafts in GPR55 cell death mechanism [30].

N-(Adamantan-1-yl)-4-ethoxy-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine; a 1,3,5-triazine derivative and strong CB2 receptor agonist, was able to reduce tumor cell proliferation in a CB independent receptor pathway [41]. The exact mechanism behind this effect remains unclear, but neither CB1 nor CB2 receptor antagonists were able to reverse it [41].

In vivo **Results**

Murine melanoma models

The benefits of ECS modulation in the treatment of this skin cancer have also been shown in *in vivo* studies with murine melanoma models. In these models, melanomas are induced by the subcutaneous inoculation of tumorous cells. *In vivo* stimulation of ECS receptors by a variety of cannabinoids was able to decrease tumor cell proliferation, tumor growth and metastasis, and increase overall survival of mice [14, 17, 19, 25, 32, 42].

Blazquez, Carracedo [17] was the first author to demonstrate the benefits of targeting the ECS *in vivo*. The author showed the peritumoral administration of WIN-55,212–2 and JWH-133 was able to reduce the growth of melanoma cells, resulting in decreased tumor volume [17]. Both cannabinoids were able to induce *in vivo* apoptosis of cancerous cells, and decreased tumor vascularization (lower vascular density) compared to controls. The efficacy of JWH-133 is relevant as it is devoid of psychoactive side effects. These are mediated by CB1 receptors in the brain [17]. As demonstrated *in vitro*, both cannabinoids decreased phosphorylation of the pRb protein. WIN-55,212–2

action was independent of immune related responses, since the antitumoral effects were also observed in nude mice [17].

Armstrong, Hill [14] later demonstrated the antitumoral action of THC and CBD in nude mice. Here, oral treatment with THC or Sativex (a 1:1 mixture of THC with CBD) decreased tumor cell proliferation, increased autophagy and apoptosis. The obtained results were compared with mice treated with vehicle solution or temozolomide (a chemotherapeutic agent) [14]. Glodde, Jakobs [19] also proved that systemic THC administration was able to reduce tumor growth in immunocompetent mice bearing CB1 and CB2 receptors. THC action was CB receptor-dependent, since tumor growth wasn't inhibited in mice lacking CB1 and CB2 receptors [19]. Histological analysis of THC treated melanomas in Glodde, Jakobs [19] study showed reduced infiltration of CD45+ immune cells (macrophages and neutrophils), believed to have a pro-tumorigenic effect. THC inhibited the growth of transplanted melanoma by CB receptors modulation and by tumor microenvironment blocking. This may explain the lack of effect seen *in vitro*. Unlike previous experiments, THC did not affect tumor angiogenesis [19].

Simultaneous administration of PEA and URB597 intraperitoneally was also able to inhibit tumor growth *in vivo* [32]. Co-treatment with PEA and URB597 reduced tumor size and weight in comparison to control treated mice. PEA and URB597 induced tumor necrosis, but no difference was seen in tumor apoptosis and angiogenesis. The lack of effect on angiogenesis suggests that treatment-induced necrosis is not due to a lack of oxygen or nutrients supply [32]. Simmerman, Qin [42] more recently demonstrated the benefit of CBD intraperitoneal injections, demonstrating a decrease in tumor growth rate in comparison with control mice. CBD treatment prolonged mice lives' [42]. CBD wasn't as effective as cisplatin (a chemotherapeutic agent) in decreasing tumor growth and increasing survival curves. Nonetheless, CBD treated mice appeared to be have a better

life quality in comparison to cisplatin-treated mice. This data suggest CBD has the potential to be a suitable adjunct for current therapeutic regimens [42].

ECS modulators have also shown antimetastatic properties *in vivo* [19, 25].

Glodde, Jakobs [19] showed that intraperitoneal WIN-55,212–2 administration in immunocompetent and nude mice reduced lung and liver metastasis, after systemic inoculation of melanoma cells [19]. Kenessey, Banki [25] also demonstrated the antimetastatic properties of ACEA. In this experiment, a spleen liver metastasis model of mice with severe combined immunodeficiency and metastatic human melanoma cells was used [25]. Intraperitoneal administration of ACEA reduced the number of liver metastasis, after melanoma cell inoculation into the spleen of mice. AEA administration was not able to inhibit liver colonization. AEA and ACEA were not able to inhibit primary tumor growth in the spleen, only liver metastasis [25].

Evidences in humans

With respect to the efficiency of cannabinoids in the treatment of melanoma in humans, no data was found in our research.

Conclusions

Recent studies have shown how targeting the endocannabinoid system may be useful in skin melanoma treatment. CB1 and CB2 receptors activation in neoplastic melanoma cells *in vitro* plays a pivotal role in growth inhibition. This is mostly accomplished by the induction of cell-cycle arrest and cell apoptosis. The classical cannabinoid receptors also play a role in the antimigratory effect induced by cannabinoids. Membrane lipid rafts and GPR55 receptor activation are also involved in cannabinoid effect on melanoma cells. Nonetheless, a significant amount of research is still needed to decipher the underlying cell mechanisms responsible for the antitumoral effects discussed.

Through the same aforementioned mechanisms, cannabinoids have also been shown to reduce tumor cell proliferation, tumor growth and to decrease metastasis *in vivo*. In spite of these results, there are still no evidences in humans.

In conclusion, the endocannabinoid system may be an interesting target of melanoma treatment. Unlike other cancers that typically affect an elder population, melanoma targets a younger demographic, which contributes to the burden of this pathology. Alternative forms of treatment are needed. More pre-clinical and clinical studies should be conducted to verify efficiency, security and tolerance of these compounds.

Bibliography

1. Biro, T., et al., *The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities.* Trends Pharmacol Sci, 2009. **30**(8): p. 411-20.
2. Rio, C.D., et al., *The endocannabinoid system of the skin. A potential approach for the treatment of skin disorders.* Biochem Pharmacol, 2018. **157**: p. 122-133.
3. Hart, S., O.M. Fischer, and A. Ullrich, *Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor.* Cancer Res, 2004. **64**(6): p. 1943-50.
4. Mounessa, J.S., et al., *The role of cannabinoids in dermatology.* J Am Acad Dermatol, 2017. **77**(1): p. 188-190.
5. Stander, S., et al., *Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin.* J Dermatol Sci, 2005. **38**(3): p. 177-88.
6. Magina, S., et al., *Inhibition of basal and ultraviolet B-induced melanogenesis by cannabinoid CB(1) receptors: a keratinocyte-dependent effect.* Arch Dermatol Res, 2011. **303**(3): p. 201-10.
7. Soliman, E., *Cannabinoids as Therapeutics for Non-Melanoma and Melanoma Skin Cancer.* Journal of Dermatology and Clinical Research, 2016. **4**.
8. Kumar, V., A. Abbas, and J. Aster, *Robbins & Cotran Pathologic Basis of Disease*. Vol. 9. 2014: Elsevier/Saunders. 1408.
9. Moreira, J., et al., *Melanoma Maligno Cutâneo: Estudo Retrospetivo de Sete Anos (2006-2012).* Acta Médica Portuguesa, 2014. **27**(4): p. 480-488.

10. Cancer.org. *Melanoma Skin Cancer Risk Factors | Melanoma Risk Factors*. 2019 [cited 2019 September 2019]; Available from: https://www.cancer.org/cancer/melanoma-skin-cancer/causes-risks-prevention/risk-factors.html.

11. Zheng, D., et al., *The cannabinoid receptors are required for ultraviolet-induced inflammation and skin cancer development.* Cancer Res, 2008. **68**(10): p. 3992-8.

12. Fund, W.C.R. *Skin cancer statistics*. 2019 September 2019]; Available from: https://www.wcrf.org/dietandcancer/cancer-trends/skin-cancer-statistics.

13. Domingues, B., et al., *Melanoma treatment in review.* Immunotargets Ther, 2018. **7**: p. 35-49.

14. Armstrong, J.L., et al., *Exploiting cannabinoid-induced cytotoxic autophagy to drive melanoma cell death.* J Invest Dermatol, 2015. **135**(6): p. 1629-1637.

15. Houghton, N.e.a., *Focus on melanoma.* Cancer Cell, 2002. **2**: p. 275-278.

16. Network, N.C.C. *Cutaneous Melanoma (Version 1.2020)*. 2019; Available from: https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf.

17. Blazquez, C., et al., *Cannabinoid receptors as novel targets for the treatment of melanoma.* FASEB J, 2006. **20**(14): p. 2633-5.

18. Carpi, S., et al., *Tumor-promoting effects of cannabinoid receptor type 1 in human melanoma cells.* Toxicol In Vitro, 2017. **40**: p. 272-279.

19. Glodde, N., et al., *Differential role of cannabinoids in the pathogenesis of skin cancer.* Life Sci, 2015. **138**: p. 35-40.

20. Ladin, D.A., et al., *Preclinical and Clinical Assessment of Cannabinoids as Anti-Cancer Agents.* Front Pharmacol, 2016. **7**: p. 361.

21. Velasco, G., et al., *The use of cannabinoids as anticancer agents.* Prog Neuropsychopharmacol Biol Psychiatry, 2016. **64**: p. 259-66.

22. Moher, D., et al., *Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.* PLoS Med, 2009. **6**(7): p. e1000097.

23. Shen, H.M. and P. Codogno, *Autophagic cell death: Loch Ness monster or endangered species?* Autophagy, 2011. **7**(5): p. 457-65.

24. Massi, P., et al., *The non-psychoactive cannabidiol triggers caspase activation and oxidative stress in human glioma cells.* Cell Mol Life Sci, 2006. **63**(17): p. 2057-66.

25. Kenessey, I., et al., *Revisiting CB1 receptor as drug target in human melanoma.* Pathol Oncol Res, 2012. **18**(4): p. 857-66.

26. Reggio, P.H., *Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown.* Curr Med Chem, 2010. **17**(14): p. 1468-86.

27. Carpi, S., et al., *AM251 induces apoptosis and G2/M cell cycle arrest in A375 human melanoma cells.* Anticancer Drugs, 2015. **26**(7): p. 754-62.

28. Grosch, S., et al., *Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors.* J Natl Cancer Inst, 2006. **98**(11): p. 736-47.

29. Paunescu, H., et al., *Cannabinoid system and cyclooxygenases inhibitors.* J Med Life, 2011. **4**(1): p. 11-20.

30. Adinolfi, B., et al., *Anticancer activity of anandamide in human cutaneous melanoma cells.* Eur J Pharmacol, 2013. **718**(1-3): p. 154-9.

31. Van Dross, R.T., *Metabolism of anandamide by COX-2 is necessary for endocannabinoid-induced cell death in tumorigenic keratinocytes.* Mol Carcinog, 2009. **48**(8): p. 724-32.

32. Hamtiaux, L., et al., *The association of N-palmitoylethanolamine with the FAAH inhibitor URB597 impairs melanoma growth through a supra-additive action.* BMC Cancer, 2012. **12**: p. 92.

33. Hasko, J., et al., *CB2 receptor activation inhibits melanoma cell transmigration through the blood-brain barrier.* Int J Mol Sci, 2014. **15**(5): p. 8063-74.

34. Ramirez, S.H., et al., *Activation of cannabinoid receptor 2 attenuates leukocyte-endothelial cell interactions and blood-brain barrier dysfunction under inflammatory conditions.* J Neurosci, 2012. **32**(12): p. 4004-16.

35. Zhao, Y., et al., *Activation of cannabinoid CB2 receptor ameliorates atherosclerosis associated with suppression of adhesion molecules.* J Cardiovasc Pharmacol, 2010. **55**(3): p. 292-8.

36. Adhikary, S., et al., *Signaling through cannabinoid receptor 2 suppresses murine dendritic cell migration by inhibiting matrix metalloproteinase 9 expression.* Blood, 2012. **120**(18): p. 3741-9.

37. De Petrocellis, L., et al., *Effect on cancer cell proliferation of palmitoylethanolamide, a fatty acid amide interacting with both the cannabinoid and vanilloid signalling systems.* Fundam Clin Pharmacol, 2002. **16**(4): p. 297-302.

38. Di Marzo, V., et al., *Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells.* Biochem J, 2001. **358**(Pt 1): p. 249-55.

39. Scuderi, M.R., et al., *The antimitogenic effect of the cannabinoid receptor agonist WIN55212-2 on human melanoma cells is mediated by the membrane lipid raft.* Cancer Lett, 2011. **310**(2): p. 240-9.

40. Osada, S., S. Saji, and K. Osada, *Critical role of extracellular signal-regulated kinase phosphorylation on menadione (vitamin K3) induced growth inhibition.* Cancer, 2001. **91**(6): p. 1156-65.

41. Yrjola, S., et al., *Synthesis, in vitro and in vivo evaluation of 1,3,5-triazines as cannabinoid CB2 receptor agonists.* Eur J Pharm Sci, 2015. **67**: p. 85-96.

42. Simmerman, E., et al., *Cannabinoids as a Potential New and Novel Treatment for Melanoma: A Pilot Study in a Murine Model.* J Surg Res, 2019. **235**: p. 210-215.

Figure Legends

Figure 1. Flow diagram of the literature search process.

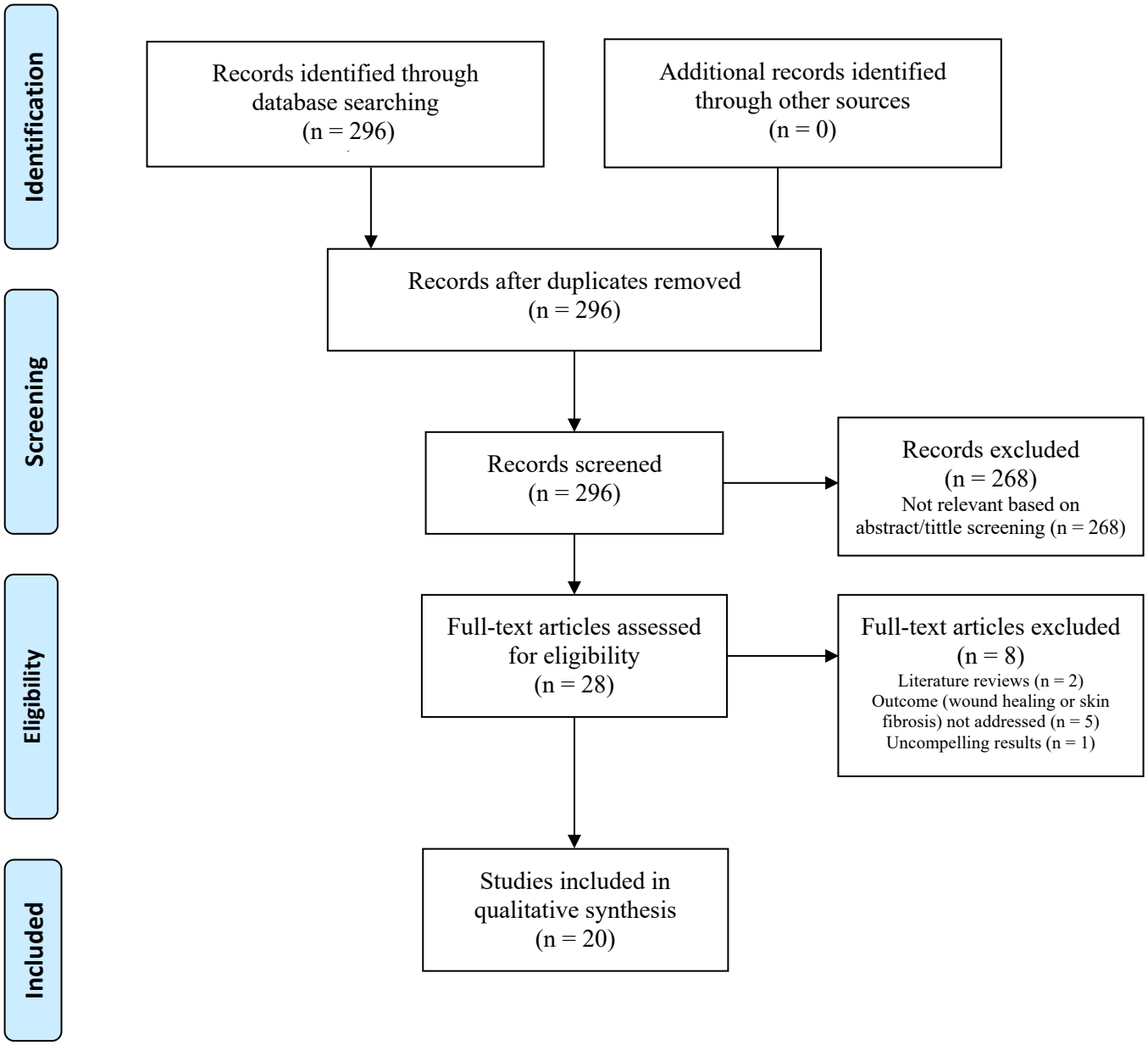

Table 1. Main results of published studies on endocannabinoid system and melanoma treatment. Cannabinoid (CB), Cannabinoid 1 receptor (CB1), Cannabinoid 2 receptor (CB2), G protein-coupled receptor 55 (GPR55), Fatty acid amide hydrolase (FAAH).

Study	Experimental Model	Administration	Cannabinoid Receptor	Other receptors	Other enzimes	Agonist	Antagonist / Inverse Agonist	Other Modulators	Results
Blazquez, C., et al. 2006 [17]	In vitro (A375 human and B16 murine melanoma cell lines)	-	CB1, CB2	-	-	THC, WIN-55, 212–2	-	-	↓ Cell growth
Blazquez, C., et al. 2006 [17]	In vivo (mice with B16 murine cells)	Peritumoral and Intraperitoneal	CB1, CB2	-	-	JWH-133 (CB2-selective), WIN-55,212–2	-	-	↓ Tumor growth ↓ Metastasic spreading
Adinolfi, B., et al. 2013 [30]	In vitro (A375 human cell lines)	-	CB1	GPR55	FAAH, COX-2	AEA	URB597 (FAAH inhibitor)	-	↓ Cell growth
Scuderi, M., et al. 2011 [39]	In vitro (COLO38, SKMEL28 and OCM1A cell lines)	-	-	Lipid Raft Complexes	-	THC, WIN-55, 212–2	-	-	↓ Cell growth
Armstrong, L., et al. 2015 [14]	In vitro (CHL-1, SKMEL28 and A375 cell lines)	-	CB1, CB2	-	-	THC	-	-	↓ Cell growth
Armstrong, L., et al. 2015 [14]	In vivo (athymic mice with CHL-1 cells)	Oral	CB1, CB2	-	-	THC, Sativex	-	-	↓ Cell growth ↓ Tumor growth
Simmerman, E., et al. 2019 [42]	In vivo (C57BL/6 mice with B16F10 murine melanoma cell line)	Intraperitoneal	-	-	-	CBD	-	-	↓ Tumor growth ↑ Survival
Kenessey, I., et al. 2012 [25]	In vitro (HT168-M1, HT199, WM35, WM983B human melanoma cell lines)	-	CB1	-	-	AEA, ACEA, Met-F-AEA	AM251 (CB1 inverse agonist)	-	↓ Cell growth ↑ Necrosis ↓ Cell migration
Kenessey, I., et al. 2012 [25]	In vivo (SCID mice with HT168-M1 cells)	Intraperitoneal	CB1	-	-	AEA, ACEA	-	-	↓ Metastasic spreading
Haskó, J., et al. 2014 [33]	In vitro (A2058 human amelanotic melanoma cell line)	-	CB2	-	-	JWH-133 (CB2-selective)	-	-	↓ Cell adhesion
Hamtiaux, L. et al. 2012 [32]	In vitro (B16 murine and MZ2-MEL.43 human melanoma cell line)	-	CB1	-	-	PEA and AEA	URB597 (FAAH inhibitor)	-	↓ Cell growth
Hamtiaux, L. et al. 2012 [32]	In vivo (mice with B16 murine cells)	Intraperitoneal	-	-	-	PEA, AEA	URB597 (FAAH inhibitor)	-	↓ Cell growth ↑ Necrosis
Glodde, N., et al. 2015 [19]	In vitro (HCmel12 and B16 murine melanoma cell lines)	-	CB1, CB2	-	-	THC	-	-	⌀ Effect
Glodde, N., et al. 2015 [19]	In vivo (mice with HCmel12 cells)	Systemic	CB1, CB2	-	-	THC	-	-	↓ Tumor growth
Carpi, S.; et al. 2015 [27]	In vitro (A375 human cell line)	-	CB1	-	-	-	AM251 (CB1 inverse agonist)	Celecoxib	↓ Cell growth
Yrjölä, S.; et al. 2015 [41]	In vitro (C8161 human cell line)	-	-	-	-	-	-	N-(Adamantan-1-yl)-4-ethoxy-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine	↓ Cell growth

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Ao meu Pai, pelo apoio incondicional e por me ajudar sempre a levantar.

À minha Mãe, por nunca ter duvidado de mim.

À Maria, por todo o carinho, apoio e motivação para nunca parar até alcançar o sucesso.

A todos os meus amigos, que são a família que escolhi e aos quais estou eternamente em dívida.

À Dr.ª Inês Sá, pela ajuda neste projeto.

ANEXOS

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AUTHOR INFORMATION PACK

TABLE OF CONTENTS

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5. Papers describing the pharmacological activities of natural compounds are considered only if they identify novel mechanisms of action.

Note that ethnopharmacological studies generally do not fall into the scope of this journal. Exceptions are made for papers addressing the mechanisms of actions or the clinical applications of worldwide-used natural substances. Clinical studies on commercially-available nutraceuticals are also taken into consideration.

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