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Notch receptors as a therapeutic target in melanoma: a narrative bibliographic review

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Abstract

Melanoma is the skin cancer with higher mortality and more and more cases are arising every year. Overexpression of Notch signaling pathway elements have already been found in primary and metastatic melanoma lineages, and directly correlated to melanoma's development, growth, angiogenesis, metastasis, and resistance to treatment. Thus, target therapy against Notch in melanoma presents a high potential for the treatment of this type of cancer. In this review we aim to perform a narrative review on melanoma's possible treatments targeting the Notch pathway. We searched literature about Notch signaling pathway inhibitors in human cutaneous melanoma published between 2000 and 2020 using MEDLINE (PubMed), LILACS (Virtual Health Library) and Cochrane Library databases. The selected articles were analyzed, summarized, tabulated, and used to produce the present narrative review. The 24 selected articles, as well as articles referenced in them, presented as targeting therapy against Notch, γ-secretase inhibitors (GSIs), primarily, but also gliotoxin, honokiol, phospholipase A2, andrographolide and monoclonal antibodies, that, however, were not directly studied in melanoma. Another therapy that indirectly interfered in the Notch signaling pathway and was found in these articles were G9a inhibitors. Analyzing the collected data, it was possible to conclude that GSIs, more extensively studied, are probably not the best option for melanoma's treatment, exceeding specific scenarios or through their concomitant use with other pathways inhibitors. The use of the other compounds, on the other hand, has greater potential, however, more studies are needed to prove its effectiveness and viability for the treatment of human cutaneous melanoma.

Key words: Melanoma; Notch receptors; Inhibitors;

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Introduction

Nowadays, even with the therapeutic advance of immunotherapy and target therapy, melanoma is still the skin cancer with the greatest mortality.1 According to the International Agency for Research on Cancer, it is expected an increase of up to 62% in the incidence of melanoma cases and an increase of 74% of deaths from this type of cancer by 2040. [2,3]

Melanoma comes from melanocytes or from their precursors as a result from the activation of oncogenes or the inactivation of tumor suppressor genes. It has an intense and complex interaction between different signaling pathways that includes mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K) - protein kinase B (Akt), phosphatase and tensin homologue (PTEN), melanocyte inducing transcription factor (MITF), Notch, among others [4]. The depth of the melanoma, which is the thickness measured from the granular layer to its deepest portion (The Breslow Depth), is the most important factor of the primary tumor prognosis [1].

The highly conserved Notch signaling pathway, from Drosophilas to humans, is essential for normal epithelial development. Its aberrant expression is directly associated with the development, the growth, the maintenance of the tumor cancer cells, the angiogenesis, the metastasis and the resistance to treatment in melanoma. It's also involved in neurodegenerative diseases, such as Alzheimer's and developmental disorders. Even though it is primarily oncogenic, Notch has already been identified as a tumor suppressor [4,5,6].

Elevated levels of Notch1-4 expression have already been associated with metastatic and primary melanoma strains, whereas, in normal melanocytes and nevi, Notch levels are low or undetectable [4,7,8], demonstrating the importance of this pathway in the development of this type of tumor.

The Notch signaling pathway in melanomas is activated by justacrine signaling, through contact between differentiated melanocytes and keratinocytes, through direct interactions between Notch receptors (Notch 1-4) and Notch ligands (Delta-like 1, 3 and 4 - ligands, Jagged-1 and 2) [9,10,11]. After the ligand - receptor interaction, cleavage of the receptor occurs by a metalloprotease, the TNF-alpha converting enzyme (TACE), releasing the extracellular domain of Notch, which will be degraded. A second cleavage happens by the γ-secretase complex, resulting in the release of the active Notch intracellular domain (NICD). The NICD translocates to the nucleus and functions as a transcription factor to influence gene expression. The main Notch target genes are genes from the Hes and Hey family, among other targets [4].

In addition to the accumulation of genetic mutations, changes in the epigenome, that is, in histones, also contribute to the onset and progression of cancer, including cutaneous melanoma. An octomer consisting of two copies of each histone (H2A, H2B, H3 and H4) is the structure in which about 146 base pairs are involved, giving rise to the nucleosome [12]. Histones have a protruding N-terminal tail, rich in lysine, which can undergo reversible post-translational covalent modifications, such as acetylation, methylation, phosphorylation, ubiquitination and sumoylation, affecting gene expression, which characterizes epigenetics. Deregulation of this process can contribute to the onset and progression of cancer, due to modifications in the N-terminal tail that alter the affinity of histones, allowing or preventing the access of transcription factors to the

deoxyribonucleic acid (DNA) that surrounds it, which can alter the transcription, replication and stability of this chromatin [13,14,15].

Histone methylation is catalyzed by histone methyltransferases (HMTs) and demethylation by histone demethylases. Methylation, which occurs in the lysine or arginine residues of the N-terminal portion of histones H3 and H4, leads predominantly to gene silencing, however, specific methylation sites can activate gene expression. In cancer, there is a change in histone methylation patterns [16, 17,18].

Among the HMTs, there is G9a, which overexpression has already been associated with different types of cancer and worse prognosis, being frequently found in metastatic and aggressive strains, including melanoma, in part, by activating the Notch1 signaling pathway through an epigenetic pathway [12,19,20,21].

Considering the effects of Notch overexpression on the development of melanoma and its role in therapeutic resistance, the manipulation of this pathway has great potential for therapeutic value. Many strategies to inhibit the Notch pathway can be used against melanoma and other types of cancer, such as γ-secretase inhibitors (GSI), monoclonal antibodies against Notch receptors or their ligands, gliotoxin, Honokiol (HNK), enzyme inhibitors methylation and so many other ways that can be explored. Thus, the present study aims to conduct a literature review on the main inhibitors of the Notch signaling pathway in cutaneous melanoma, an important potential therapeutic target.

Materials & Methods

In the MEDLINE (via PubMed), Lilacs (via *Biblioteca Virtual em Saúde*) and Cochrane Library databases, searches for articles using Notch signaling inhibitors in human cutaneous melanoma between the years 2000 and 2020 were performed, thus excluding studies with uveal melanoma and animal cell lines.

For the search, the following descriptors, combined or individually, were used: “Melanoma”, “Notch”, “Notch1”, “Notch2”, “Notch3”, “Notch4”, “Notch antibody”, “Gliotoxin”, “Honokiol”, “Phospholipase A2”, “Notch inhibitor”, “G9a”, “GSI” and “Gamma-secretase inhibitor”, on MEDLINE database and Chochrane Library. For the search on Lilacs, the keywords were used, not only in English, but also in Spanish. The MeSH terms used were “Melanoma”; “Notch receptors”; “Notch proteins”; “honokiol”, “gliotoxin”, “phospolipases A2” and “UNC0642”.

Each set of terms used were tabulated to determine the number of “results”; “selected articles after reading the abstract" and “selected articles after reading the full text". Search strategy in each database is described in table 1.

Database	Search strategy
Medline (Pubmed)	Melanoma [title] and (Notch [title] or Notch1[title] or Notch2 [title] or Notch3 [title] or Notch4 [title])
LILACS (Bireme)	Melanoma and (Notch or Notch1 or Notch2 or Notch3 or Notch4)
Cochrane Library	Melanoma and (Notch or Notch 1 or Notch 2 or Notch 3 or Notch 4)
Medline (Pubmed)	Melanoma[title] and (Notch proteins or Notch Receptors) and Notch antibody
LILACS (Bireme)	Melanoma and (Notch proteins or Notch Receptors or proteína Notch or receptores Notch) and (Notch antibody or anticuerpo Notch)
Cochrane Library	Melanoma and (Notch proteins or Notch Receptors) and Notch antibody
Medline (Pubmed)	(Melanoma [Title]) and Honokiol
LILACS (Bireme)	Melanoma and Honokiol
Cochrane Library	Melanoma and Honokiol
Medline (Pubmed)	(Melanoma [Title]) and Gliotoxin
LILACS (Bireme)	Melanoma and Gliotoxina or Gliotoxin)
Cochrane Library	Melanoma and Gliotoxin
Medline (Pubmed)	(Melanoma [Title]) and Phospholipase A2
LILACS (Bireme)	Melanoma and (Fosfolipasas A2 or Phospholipases A2)
Cochrane Library	Melanoma and Phospholipase A(2)
Medline (Pubmed)	(Melanoma [Title]) and (Gamma-secretase inhibitor or GSI)
LILACS (Bireme)	Melanoma and (Gamma-secretase inhibitor or GSI or inhibidor de gamma-secretasa)
Cochrane Library	Melanoma and amma-secretase inhibitor
Medline (Pubmed)	Melanoma[title] and (Notch inhibitor or Notch antagonist)
LILACS (Bireme)	Melanoma and (Notch inhibitor or Notch antagonist or inhibidor Notch or antagonista Notch)
Cochrane Library	Melanoma and (Notch inhibitor or Notch antagonist)
Medline (Pubmed)	G9a and melanoma
LILACS (Bireme)	G9a and melanoma
Cochrane Library	G9a and melanoma
Medline (Pubmed)	Melanoma [title] and (Notch receptors or Notch proteins) and UNC0642
LILACS (Bireme)	Melanoma and (Notch proteins or Notch Receptors or proteína Notch or receptores Notch) and UNC0642
Cochrane Library	Melanoma and (Notch receptors or Notch proteins) and UNC0642

Table 1- Search strategies used in each database

Results

With the sum of the results of all searches, 711 articles were found, of which, after reading the abstract, 106 articles remained. Finally, after reading the full text, 24 articles were kept. All of them were analyzed, summarized, tabulated and used for the production of the present narrative review, considering the analysis of all authors.

Although most of the articles studied the action of GSIs on Notch signaling and cell viability, presenting more data and possible discussions, the number of articles that used other diverse compounds (such as Honokiol and Gliotoxin) were considerable, what had a great impact in this review, since those studies showed important information about the effect of these compounds on Notch signaling pathway and cell viability. Despite being cited as one of the main forms of target therapy for Notch signaling, no experimental studies were found using anti-Notch antibodies in melanoma.

There was a scarcity of studies of Notch signaling inhibitors in melanoma, in contrast to their studies in other types of cancer, mainly regarding the use of anti-Notch antibodies, even though, as will be presented, these inhibitors present great efficiency in inhibiting Notch signaling and cell viability of melanoma.

Gamma-secretase inhibitors (GSI)

Since the γ-secretase complex is essential for the second cleavage of Notch receptors, originating its active form, NICD, it has been studied as a target for therapy in melanomas, using GSIs.

Using GSI N- [N- (3,5 - difluorophenacetyl-L-alanyl)] - S-phenylglycine t-butyl ester (DAPT) to inhibit the Notch pathway in melanoma cells, Balint et al (2005) noted that this GSI was able to reduce cell growth rates in both primary tumor cells and metastatic cells. However, the metastatic cells were inhibited only with the use of higher doses (1 μM) of DAPT, what showed a certain difference in sensitivity to treatment with this GSI between these melanoma cells, especially at low doses. This was associated with the fact that Notch1 promotes the progression of primary tumors by acting on the expression of β-catenin, which was shown to be elevated in these cells, but not in metastatic cells. In addition, β-catenin was also affected, with its levels reduced in primary cells [22]. However, it is interesting to note that, in a later study, Bedogni et al (2008) reported that, differently from what was previously demonstrated, β-catenin apparently was not affected by Notch 1 inhibition, while cyclin D1 appeared inhibited, suggesting that cyclin D1, which would be regulated by β-catenin, undergoes direct control of Notch1 activity and that its inhibition is associated with reduced tumor cell growth [23].

The difference in sensitivity reported by Balint et al (2005) was also noted by Bhat et al (2006). When studying the expression of microtubule associated protein 2 (MAP2) in melanomas, which has the function of stabilizing microtubules and, when elevated, was associated with higher disease-free survival rates, they suggested that the Notch 1 pathway is associated, through Hes 1, with the regulation of the MAP2 gene and that primary melanoma cells are more sensitive to inhibition of Notch1 and to activation of the MAP2 promoter, using GSI DAPT, than metastatic cells [24].

The progression of several solid tumors, including melanomas, is associated with hypoxia and Akt activation. Analyzing the role of the Notch1 pathway in the interaction between these two factors, melanocytes expressing Akt, cultured in

hypoxic atmosphere, were treated with GSI DAPT and showed a reduction in colony formation, due to the inhibition of Notch activation, and, after xenograft in mice, there was a reduction in tumor growth, in a dose-dependent manner, with the application of topical GSI DAPT [23]. However, when considering therapeutic options for melanoma, it is also important to note that Liu et al (2017) subsequently suggested, in their study, that melanoma cells cultured under hypoxia are less susceptible to various therapeutic agents, including GSI DAPT, when compared to cells cultured under normal conditions [25].

Although most of the studies found studied Notch1, Lin et al (2016) observed that the inhibition of Notch4, with the use of GSI DAPT, was able to reduce metastatic properties of melanoma cells, suggesting that GSIs may be an option in the strategy therapy to inhibit metastases in these tumors and that Notch4 can be a target for treatment, although further investigations are needed to confirm that [26].

Although studies, such as those already mentioned, have associated the use of GSIs with tumor, the real efficiency of the isolated use of GSI DAPT, in a long run term, does not seem to be sufficient. Therefore, GSI DAPT does not seem to be an effective therapeutic option in the treatment of tumors, according to a study conducted by Key Ghobadi et al (2020), where it was shown that, even though in a short term the use of DAPT has reduced the formation of spheres and colonies in metastatic melanoma cells, its use, in a long term, was associated with an increase in these formations, with a greater number of cells, and may even induce drug resistance. Likewise, the study reported that GSI DAPT is able to reduce the size of tumors in mice, only transiently. When trying to understand the mechanism for this, it was observed that the use of DAPT, in a short time (48h), reduced the expression of genes downstream Notch (Notch1, Notch2, Hes1), while genes from the Wnt pathway (CTNNB1 and c-myc) and intermediate genes (AXIN1, CSNK2A3 and CEBPA2) showed increased expression. With the interruption of this treatment, the expression of Notch returned to normal or even increased, while that of the others declined. The use for a longer period (6 days) showed that the expression of all these genes was reduced, but when the treatment stopped, it returned to normal and the melanoma cells became more aggressive, with an increase in the formation of spheres and colonies [27].

Another type of GSI studied for the treatment of melanomas was GSI RO4929097. In a study conducted by Huynh et al (2011), this drug was shown to be able to interfere in the tumorigenicity of melanoma cells, acting in the Notch pathway and reducing the expression of some of its downstream genes, such as HES1. It was associated with a reduction in the tumor initiation ability of melanoma cells, as well as interfering with their metastatic abilities and the growth of existing tumors. However, it should be noted that, although it has shown activity against primary and metastatic tumor cells, there is no universal response to this drug, since the sensitivity to it varies between melanoma cell lines [8].

Nair et al (2013) studied the susceptibility of melanoma cells to the action of GSI RO4929097 concerning their PTEN status. PTEN, which is one of Notch1's transcriptional targets, is a tumor suppressor gene which encodes a lipid phosphatase associated with negative regulation of the PI3K-Akt pathway, related to cell growth, cell survival and cell cycle progression. The study suggests that the GSI in question inhibits melanoma cell growth in a PTEN-dependent manner, since cells with null or mutant PTEN did not present apoptosis or

senescence, while cells with PTEN overexpression showed greater cellular sensitization to the action of GSI RO4929097, proposing that the loss of this gene, found in about a quarter of BRAFV600E mutant melanoma cells, may be associated with resistance to this treatment and it could be interesting to select patients sensitive to the use of this and other GSIs in order to develop efficient drugs [28].

Despite presenting a clinical response in two patients with melanoma in a phase I clinical trial [29], in a phase II clinical trial, the use of GSI RO4929097 in unselected metastatic melanoma cells, although well tolerated, presented a minimal response to the recommended doses and schedule for this type of study. Lee et al. (2015) suggests that the autoinduction of the metabolism of this drug and that gastrointestinal toxicities, which limits the dosage not only of this GSI, but also of others, may not place this class as the best option for the development of new medications. Thus, although the study does not recommend the isolated use of GSI RO4929097 for unselected patients, they claim that inhibition of the Notch pathway is important in antitumor activity in melanomas, and that alternatives should be sought to achieve it, such as the use of monoclonal antibodies [30].

Despite the existence of MAPK inhibitors available for the treatment of patients with BRAFV600E mutant melanoma, about 30% of them do not respond to these inhibitors. Searching for a strategy for its treatment, Krepler et al (2016) noted that, while GSI RO4929097 and MAPK inhibitor SCH772984 used alone did not affect non-responsive cells, their combination was able to reduce the viability of these cells and induce apoptosis, concluding that the blocking of Notch signaling by GSI potentiated the action of the MAPK inhibitor, so that they were used together to inhibit tumor growth. However, at the study doses, they were unable to cause tumor regression [31].

Using (S, S) - 2- [2- (3,5-Difluorophenyl) -acetylamino] -N - (1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo [e] [1,4] diazepin-3-yl) - propionamide, Skarmoutsou et al (2018) determined that this GSI can reduce cell viability and proliferation, causing a reduction in Notch1 levels. This efficiency is probably due to the modulation of Forkhead box protein P3 (FOXP3) by Notch 1, what occurs in two ways: cooperatively with the Transforming Growth Factor-β1 (TGF- β1), which also positively modulates Notch expression, and directly elevating FOXP3 transcription. FOXP3 high expression is a marker of aggression and metastasis in melanoma [32].

The use of GSI dibenzazepine (DBZ), RO4929097 and Lapatinib (EGFR / ERBB2 inhibitor) in human melanoma metastatic cell lines, in general, showed high efficiency in reducing cell growth both in cells with mutated BRAF and in wild BRAF cells. However, the combined use of one or the other GSI with Lapatinib showed better results in inhibiting metastatic melanoma, compared to the use of these inhibitors alone, reducing cell viability by 90%, hindering tumor growth and inducing apoptosis, which indicates a potential action in tumor regression. This is due, in part, to inhibition of AKT phosphorylation and inhibition of the cell survival pathway that includes nuclear factor kappa B (NFκB). It is important to highlight that the inhibition of NFκB plays an important role in the inhibition of histone demethylase cells JARID1B (+), in which GSIs and Lapatinib also act [33,34].

Finally, GSI I showed promising results, showing pro-apoptotic activity in melanoma cells, by inhibiting the four Notch receptors and consequent positive regulation of Bcl-2-like protein 11 and protein 1 induced by phorbol-12-myristate-13 -acetate (Noxa), but not in normal melanocytes, which suffered cell

cycle arrest in the G2 / M phase [4]

Monoclonal antibodies targeting Notch receptors (moAb)

Monoclonal antibodies are a class of developing agents targeting both Notch receptors and their ligands. Monoclonal antibodies, such as OMP-59R5 (tarextumab), OMP-21M18 (demcizumab), REGN421 / SAR153192, OMP-52M51, showed antitumor effect in several types of tumors, however, no study has been found to evaluate their effect on melanoma [35,36].

It was observed as an advantage, in relation to GSIs, the possibility of directing the inhibition only to Notch1 or Notch2, which reduces the toxic effects when compared to the concomitant inhibition of the two receptors[36].

G9a inhibitors

Inhibition of G9a expression has been shown to affect the expression levels of Notch1 and Hes1, which prevents Notch1 from activating the PI3K-Akt pathway, which contributes to melanoma cell proliferation and migration. The mechanisms for this effect have not yet been fully established, but this is probably because G9a must inhibit the expression of certain Notch1 regulatory genes. The use of UNC0642, a G9a inhibitor, in vitro reduced the viability and invasion capacity, in addition to inducing apoptosis in melanoma cells, and in vivo, it was able to inhibit cell growth [21].

G9a is a histone lysine methyltransferase that catalyzes the methylation of histones H3 lysine 27 (H3K27) and H3 lysine 9 (H3K9), in addition to some proteins such as chromatin-remodeling factor-Pontin, tumor protein p53 and myoblast determination protein 1 [21]. Methylation of H3K9 is directly associated with cancer progression, leading to transcriptional repression of tumor suppressor genes, in the case of dimethylation, or leading to gene activation, in the case of monomethylation [21,37,38,39]. It consists of the repetition of ankyrin that recognize H3K9, a self-methylation site at the N-terminal end and a region responsible for its enzymatic activity, the catalytic SET domain [40].

Hypoxia, important for the development of metastases, is associated with reduced expression of cell adhesion molecules, which facilitates the epithelium-mesenchymal transition. Through hypoxia there is an increase in G9a activity, increasing the global methylation of H3K9, which inhibits the expression of adhesion factors, such as E-cadherins, contributing to the metastatic process [19,20,41]. For this reason and the presence of its increased expression in metastatic strains, G9a is considered a key factor for the occurrence of metastasis.

Considering the role of G9a in oncogenesis, several G9a inhibitors have been developed and have been tested in vivo and in vitro, seeking the discovery of more potent and selective inhibitors for the enzyme. Thus, Liu et al. (2013) synthesized the G9a and LPG inhibitor, UNC0642 from UNC0638, which demonstrated high selectivity and low cell toxicity, when tested in different cell lines that included breast, prostate, osteosarcoma and epithelioid carcinoma strains, in addition to one improved pharmacokinetics in vivo.[38,42,43].

Other inhibitors

Several natural compounds, such as gliotoxin, phospholipase A2 derived from the venom of the

serpent species *Daboia siamensis* (dssPLA2), Honokiol and Andrografolide (Andro), proved to be efficient in interacting with the Notch pathway and to inhibit the development of melanoma.

Gliotoxin is a secondary metabolite of fungi of the genus *Aspergillus*, inhibitor of the canonical transactivation of Notch2 / recombination signal binding protein for immunoglobulin kappa J (CSL). *In vitro* study, Hubmman et al. (2017) studied the effects of gliotoxin in human melanoma cell lines, hepatocellular carcinoma (HCC), pancreatic cancer and breast cancer, comparing it with GSI DAPT. In these cell types, the Notch2^{NICD} / CSL complex was not sensitive to DAPT, but gliotoxin was completely able to block its formation. Experiments with HCC cells have shown that gliotoxin is efficient in blocking the formation of the Notch2 / CSL complex and inducing apoptosis in positive intracellular Notch2 cells, while DAPT has almost no effect, probably because there are non-canonical forms of Notch expression that are independent of γ-secretase for processing and because Notch2 / CSL nuclear complexes can be relatively stable, which should also explain their effects on melanoma. *In vivo*, in xenotransplanted mice with human melanoma strains, a one-day dose schedule of gliotoxin was well tolerated without any side effects limiting the study and it was able to efficiently reduce tumor volume in both early and advanced stages [44].

Another component, the low molecular weight enzyme dssPLA2 allowed Notch inhibition in melanoma cells, leading to cell death. *In vitro* experiments showed that, in a concentration-dependent manner, dssPLA2, in 24 hours, did not alter the concentrations of Notch1, 2 and 3, or BRAFV600E, but induced cytotoxicity, apoptosis and inhibited cell migration. However, the study observed that, after 72 hours of incubation, genetic damage occurred, with significant reduction in the expression of Notch1 and BRAFV600E, which suggests that these genes are associated with the induction of cytotoxicity and the consequent cell death by dssPLA2 in melanoma cells. These effects, added to the fact that the enzyme did not show cytotoxicity in normal fibroblasts, make it a potent candidate in the treatment of melanoma [45], requiring further studies on its efficiency and effect.

HNK, is a biphenolic compound from a species of the magnolia plant native to China, which has been studied as a possible treatment in several types of cancer, such as breast, skin, ovary, prostate cancers, among others[46,47,48]. In melanoma studies, HNK has been associated with attenuation of the AKT / mammalian target of rapamycin (mTOR) pathway and Notch signaling pathway, resulting in reduced cell proliferation, autophagy, halted cell cycle and inhibition of melanoma stem cells, important in tumor growth, angiogenesis and therapeutic resistance, being associated with increased expression of Notch receptors [47,48]. Despite Kaushik et al. (2012) have found no evidence of apoptosis induction, other studies have attested that HNK is capable of inducing apoptosis through interaction with glucose-regulated protein 78 [46,47].

As for its performance in the Notch pathway, HNK acts by inhibiting the expression of the TACE and γ-secretase complexes, which act in the cleavage of Notch receptors and release its active NICD domain.[47,48] The treatment of melanoma cell lines with HNK resulted in reduced levels mainly of Notch2, Notch1 and the target genes of the Notch pathway, Cyclin D1, which acts in the passage of phases of the cell cycle, and Hes-1, resulting in a significant reduction of viability, clonogenicity and melanosphere formation, inducing autophagy, but not apoptosis, and stopping the cell cycle in G0 /

G1. When compared to the GSI DAPT and DBZ, HNK was more efficient in inhibiting cell proliferation and inducing death, but the combined treatment of HNK with GSI was more efficient in reducing the cleaved Notch2 expression than the treatments with each drug alone [48]. Thus, HNK demonstrated great efficiency in inhibiting melanoma cells and mainly melanoma stem cells, in part, by inhibiting the Notch signaling pathway [47,48].

Another compound studied for the treatment of melanoma was Andro, derived from the *Andrographis paniculata* plant, which reduces cell growth, impairs the epithelial-mesenchymal transition, which favors metastasis, and reduces angiogenesis in melanoma cells prominin-1 (CD133) (+), in addition to also acting on melanoma stem cells (CD133) (+) and suppressing lung metastasis. These effects were associated with Andro's ability to nullify the Notch1-mediated CD133-dependent p38 MAPK activation pathway [49].

Discussion

Notch overexpression was directly associated with the development, growth, angiogenesis, metastasis and therapeutic resistance in melanoma, in addition to the maintenance and development of melanoma stem cells, therefore, Notch signaling pathway inhibition has a great therapeutic potential for the treatment of this type of skin cancer [6,36].

Targeted therapy drugs are more effective and have fewer toxic effects if compared with conventional therapies. The target therapy against Notch has shown as a side effect gastrointestinal toxicity, however this problem has been circumvented by strategies such as discontinuous dosage, corticosteroid therapy and the development of more selective drugs [35].

Different cell types have shown different sensitivities to treatment with GSI DAPT, some studies have shown greater effect on primary rather than in metastatic cells [22,24], and others have identified GSI DAPT as a potential therapy for inhibiting metastasis in melanoma [26]. However, the long-term use of DAPT proved to be inefficient, showing to increase the aggressiveness of melanoma and its resistance to treatment [27]. These data demonstrate that GSI DAPT is probably not the best option for melanoma's treatment targeting the Notch signaling pathway.

Different sensitivities between cell lines were also present with the GSI RO4929097, which proved efficiency depending on the cell line[8] and the higher expression of PTEN [28]. Even though it demonstrated clinical response in two patients in a phase I clinical trial, its isolated use proved to be ineffective in a phase II clinical trial, in which it demonstrated minimal response and high gastrointestinal toxicity [29,30]. Experimental studies have identified better results in its association with other inhibitors, such as MAPK inhibitors [31] and EGFR / ERBB2 inhibitor [33,34].

Other studies, which analyzed different varieties of GSIs, attested their high efficiency, correlating their ability to reduce the viability of melanoma cells to the interaction of Notch with other pathways and cellular components such as FOXP3, BH3, β-catenin and cyclin D [4,22,23,32]. The effect of GSIs was shown to be dependent on the tumor microenvironment. Hypoxia cells, despite being affected by DAPT [23], are less susceptible to therapeutic agents [25], for example.

Considering that there are non-canonical forms of the Notch signaling pathway that do not involve γ-secretase[44] and that the efficiency of GSIs depends on several factors that include genetic differences and the tumor microenvironment, GSIs became unattractive as therapies. However,

these compounds may be a good option for use in precision medicine, an area that considers the genetic differences between individuals when prescribing specific treatments, which may have more or less therapeutic efficiency or toxic effects in different populational groups, or, still, in association with inhibitors of other pathways that can potentialize its effect in the inhibition of melanoma cells.

Furthermore, when compared to other compounds such as Gliotoxin and HNK, GSIs have been shown to be less effective in inhibiting melanoma cells [44,48]. These compounds, dssPLA2 [45] and Andro [49] were shown to be potential candidates for the treatment of melanoma by inhibiting the Notch signaling pathway, inhibiting melanoma cells and even reducing tumor volume, also presenting low toxicity. Although studies of monoclonal antibodies have not been found as a target therapy for Notch in melanoma, its studies in other types of cancers demonstrated that they have lower toxicity if compared with GSI .[35,36].

Methyltransferase inhibitors, such as G9a inhibitors, interfere with the expression of the Notch signaling pathway and have shown promising results with high therapeutic potential, especially in cases where the Notch1 pathway is involved, within a broader concept of precision medicine. This concept encourages the study of other substances, such as UNC0642 or its derivatives, with greater liposolubility, greater power for cellular availability and less toxicity [21,38].

Conclusion

It is certain that the Notch signaling pathway can contribute to the appearance and progression of melanoma, therefore, the inhibition has relevant potential for the treatment of this type of cancer. Considering what was exposed in this review, GSIs are not the best option, except for specific scenarios, which can be determined by precision medicine and pharmacogenetics, or their concomitant use with inhibitors from other pathways. The use of other compounds such as Gliotoxin, dssPLA2, HNK, Andro G9a inhibitors and moAb have greater potential as target therapies for Notch in the treatment of melanoma. The findings of interference of G9 inhibitors on the viability, proliferation and metastasis of melanoma, stimulate the study of other substances, such as UNC0642 or derivatives of it, with greater liposolubility, greater availability and less cellular toxicity. However, more studies, especially in vitro (cell culture), in animals, and in humans (phase I and II) need to be done to attest Notch inhibition efficiency as a therapy for melanoma.

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