Inhibition of methylation and notch receptors in cutaneous melanoma

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ABSTRACT
Cutaneous melanoma is skin cancer that originates from melanocytes or their precursors, melanoblasts and stem cells, being a cancer with high mortality. It is considered the most serious type of skin cancer because of its ability to spread quickly to other parts of the body (Shain 2016). Excessive exposure to ultraviolet radiation from the sun (a factor modifiable by human action) is its main external cause, with heredity predominating as the main cause in about 8 to 10% of cases. This includes prolonged exposure to the sun without adequate protection as well as the use of artificial tanning beds, which emit radiation similar to that of the sun.

Keywords: Methylation, Cutaneous melanoma, Cancer.

1 INTRODUCTION

Cutaneous melanoma is skin cancer that originates from melanocytes or their precursors, melanoblasts and stem cells, being a cancer with high mortality. It is considered the most serious type of skin cancer because of its ability to spread quickly to other parts of the body (Shain 2016). Excessive exposure to ultraviolet radiation from the sun (a factor modifiable by human action) is its main external cause, with heredity predominating as the main cause in about 8 to 10% of cases. This includes prolonged exposure to the sun without adequate protection as well as the use of artificial tanning beds, which emit radiation similar to that of the sun.

Immunotherapy and targeted therapy have shown remarkable results in the treatment of advanced cutaneous melanoma. Some patients experience a lasting response to immunotherapy, with tumors significantly decreasing in size or even disappearing completely. Three classes of immunotherapies are available for clinical use: inhibitors of CTLA4 receptors (cytotoxic T-lymphocyte-associated protein 4), inhibitors of PD1 receptors (programmed cell death protein 1) or PDL1 protein, and inhibitors of LAG3. These receptors are present on T lymphocytes (Qin 2023).

Targeted therapy is another important approach in the treatment of advanced cutaneous melanoma. It inhibits specific molecules or signaling pathways that are involved in the growth and proliferation of melanoma cells. They can be used when the melanoma has mutations in the BRAF gene. About 80% of these mutations are BRAFV600E, and BRAFV600k from 5 to 30%. There are two main types of targeted therapy in cutaneous melanoma and they are most often used together: BRAF inhibitors (vemurafenib, dabrafenib, and encorafenib) and MEK inhibitors (trametinib, cobimetinib, and binimetinib). Unfortunately, most patients initially respond well and then fail to respond due to the development of resistance mechanisms. The association of immunotherapies with targeted therapy is widely used in an attempt to overcome resistance (Lopes 2022). However, the response to immunotherapy and targeted therapy varies from person to person, and not all patients are successful. About 60% of these patients develop resistance and their disease progresses. It is necessary to find new forms of treatment.

In the human genome there are approximately 23,000 genes and they encode approximately 100,000 proteins. These proteins can be altered by post-translational (epigenetic) modifications, such as sumoylation, phosphorylation, acetylation, ubiquitination and methylation of DNA and histones, proteins that form the structure around which DNA is wrapped. Epigenetic changes are divided into four levels of regulation of gene expression: DNA methylation, histone modification, chromatin remodeling and regulation of non-coding RNAs; the deregulation of lysine methyltransferase enzymes are associated with tumor initiation, invasion and development of metastases, changes in the immune microenvironment and drug resistance. These modifications regulate the ability of transcription factors to access underlying DNA and which impact transcription, replication, and chromatin stability (Bates 2021, Harel 2021, Moran 2021).

Histone methyltransferases have been the subject of intensive study in drug development. Understanding how these enzymes regulate chromatin structure and gene expression has led to the development of targeted therapies known as histone methyltransferase inhibitors. These inhibitors aim to modulate the activities of these enzymes, potentially altering gene expression patterns in diseases such as cancer.

- The two most important classes of methyltransferases are DNA methyltransferases and protein methyltransferases. The former are enzymes that add methyl groups to the nitrogenous bases of DNA. This has a significant impact on the regulation of gene expression, as DNA methylation can silence gene activity. This is because hypermethylation of gene promoter regions can lead to inhibition of gene transcription and is associated with pathological processes, including cancer. Protein methyltransferases are enzymes that add methyl groups to amino acid residues in proteins. A well-known example is the methylation of lysine and arginine to histones, proteins that help organize DNA into

chromatin. The most extensively studied histone methylation sites include histone 3 on lysine 4 (H4K4), histone 3 on lysine 9 (H3K9), histone 3 on lysine 27 (H3K27), and histone 4 on lysine 20 (H4K20) (Martin 2005, Nacev 2019). Methylation of lysine residues on histones H3 and H4 can have varying effects, depending on the specific site of methylation. For example, lysine 4 methylation of histone H3 (H3K4) is generally associated with more open and active chromatin regions where gene expression is allowed. On the other hand, lysine 9 methylation of histone H3 (H3K9) is associated with more compact chromatin regions and gene silencing. Histone lysine cleavages are associated with different chromatin states with specific implications for gene regulation:

- • H3K4 (Histone H3 lysine 4): Histone H3 lysine 4 methylation is often associated with more accessible and active regions of chromatin where gene transcription is more likely to occur. This methylation is generally seen as a marker of active promoter and enhancer regions of genes.
- H3K9 (Histone H3 lysine 9): Histone H3 lysine 9 methylation is typically associated with tight chromatin regions and gene silencing. This is because H3K9 methylation recruits proteins that promote the formation of heterochromatin, a densely packed form of chromatin that tends to silence transcription. H3K9 is initially methylated by G9a dimers (EHMT2 gene) and GLP (EHMT1 gene) to form activator H3K9 monomethylation which is further methylated by G9a to form repressive H3K9 dimethylation (H3K9me2). G9a directly contributes to dimethylation and subsequent trimethylation in H3K9 throughout the genome.H3K27 (lisina 27 da histona H3): a metilação da lisina 27 da histona H3 também está associada ao silenciamento gênico. É frequentemente encontrado em regiões de cromatina que regulam o desenvolvimento celular e a diferenciação.

Dysfunctions in methyltransferase activities can be associated with a variety of diseases, including cancer, genetic disorders and neurodegenerative diseases. Therefore, the study of methyltransferases and methyl modifications plays an important role in scientific research and medicine, in addition to understanding how these enzymes regulate cellular processes, it can lead to the development of new targeted therapies, including epigenetic treatments for cancer and other diseases. related to gene regulation. Histone methyltransferases (HMTases) are a group of enzymes that catalyze the addition of methyl groups (-CH3) to specific amino acid residues on histones, which are fundamental proteins in the structure of chromatin.

Histone lysine methyltransferases (KMTs), also known as histone lysine methyltransferases (HKMTs), are a group of enzymes that catalyze the addition of methyl groups (-CH3) to lysine residues on histones, which are the proteins that help organize the DNA in chromatin. Histone methylation is a fundamental epigenetic modification that plays an important role in the regulation of gene expression

and formation of chromatin structure. Dysregulation of histone lysine methyltransferase is associated with tumor initiation, invasion and metastasis, resistance to drugs and the immune microenvironment (Liao 2023).

KMTs work by transferring a methyl group from a methyl donor (usually the coenzyme S-adenosylmethionine, or simply SAM) to lysine residues present on histones. SAM is formed from methionine and ATP, a process that produces phosphate and pyrophosphate. After transfer of the methyl group, SAM is converted to S-adenonsilhomocysteine. This modification can occur at different positions of lysine and have varying effects on the regulation of gene expression and cell activity. Simultaneous binding of SAM to the post-SET flexible domain stimulates folding of the domain and directs the binding of the remaining peptide residues. This domain can close to complete the substrate binding site and allow catalysis to take place.

Since KMTs have shown promise in research, many studies are exploring the use of histone methyltransferase inhibitor KMTs as potential cancer treatments. That's because KMT inhibitors can be combined with other therapies such as chemotherapy, immunotherapy and targeted therapy to increase the effectiveness of treating many types of cancer. Precisely because epigenetic modifications play an important role in regulating gene expression in cancer cells, researchers are evaluating how specifically inhibiting KMTs can affect tumor growth, cell proliferation, and sensitivity to other treatments.

An example of this are the lysine histone methyltransferase (KMTs) inhibitors, which are a class of compounds that target the KMT enzymes, which catalyze the methylation of lysine residues in histones. These inhibitors were developed with the aim of modulating the activity of KMTs and, consequently, the epigenetic modifications in histones. This may have implications for the regulation of gene expression and associated cellular processes, including development, cell differentiation and disease.

Biochemical studies show that G9a and GLP have the same substrate specificity on histones. And recent publications have identified G9a as a functional oncogene in melanoma, either through recurrent activating mutations in the SET domain or through amplification of the genomic locus. Depending on the position of the modified residue, histone methylation can either suppress (H3K9, H3K27) or elevate (H3K4) gene expression. Together, histone modifications and DNA methylation tightly regulate chromatin accessibility within cells (Flesher 2021, Haeb 2021, Fath 2022).

During hypoxia, G9a activity increases, leading to an increase in global histone H3K9 methylation. As hypoxia is considered an important factor in the development of metastases, the acquisition of cell motility under hypoxic conditions has been correlated with a reduction in the expression of cell adhesion molecules. How G9a inhibits the expression of cell adhesion factors such as E-cadherin and epithelial cell adhesion molecules. The correlation between G9a-mediated

repression of cell adhesion molecules and increases in their activity during hypoxia strongly supports the direct involvement of G9a in the metastatic pathway.

G9a is a lysine methyl transferase enzyme responsible for the monodimethylation and dimethylation of histone H3 lysine 9 (H3K9), a reversible modification usually associated with the silencing of transcriptional genes. G9a overexpression is associated with a poor prognosis in different types of cancer, including melanoma, by upregulating the Nocth1 signaling pathway, silencing tumor suppressors, or activating epithelial-mesenchymal transition programs) (Casciello 2015, Fan 2015, Dang 2021 , Son 2021, Liao 2023).

Among the various preclinical studies, we highlight one where the G9a inhibitor UNC0638 combined with cisplatin for head and neck squamous cell carcinoma showed the ability to reduce resistance to cisplatin (Liu 2017) and another study, still in progress, associates EZH2 inhibitor (tazemetostat) with BRAF and MEK inhibitors for treatment of advanced melanoma (NCT05152459) (Liao 2023).

G9a inhibitors can inhibit tumor growth by blocking cell cycle progression and triggering apoptosis or inducing autophagic cell death (Cao 2019, Liao 2023). Furthermore, G9a plays a significant role in heterochromatin formation, DNA methylation, transcriptional silencing, proliferation, apoptosis, differentiation and mobility of tumor cells (Kato 2020).

A clinical trial funded by the European Union focused on the development of molecular inhibitors of the enzyme histone lysine methyltransferases (HKMT) as potential therapeutic agents for the treatment of cancer. The researchers used a class of derivatives of the 2,4-diaminoquinazoline molecule, which are known to act as competitive inhibitors of methyltransferases. After synthesizing a library of 2,4-diaminoquinazolines and evaluating the compounds through enzymatic assays and cell-based assays, different inhibitors were identified with IC50 values (50% of the maximum inhibitory concentration) of up to 1 µM. In cell-based assays, inhibitors increased mRNA levels of genes known to be silenced by activation of HKMTs. One of the inhibitors showed activity in an experimental mouse model with MDA-MB-231 xenografts (breast cancer). In 2020, the FDA approved the first KMT inhibitor, tazemetostat for the treatment of epithelioid sarcoma and follicular lymphoma (Hoy 2020, Liao 2023).

Currently, three compounds have been widely used as G9a inhibitors, BIX01294 and UNC0638 being used in vitro, and UNC0642 tested in preclinical models in vivo, due to their more favorable pharmacokinetics, better half-life, while maintaining high selectivity and low cellular toxicity; both G9a and GLP contain ankyrin repeats to recognize these methylation residues and a SET domain that is required for catalytic activity that utilizes SAM, the methyl radical donor (Flesher 2021). UNC0642 was discovered to be a potent inhibitor of G9a and GLP, with low toxicity and high selectivity, making it suitable for animal studies (Liu 2013). In this sense, the compound UNC0642 acts as a competitive

inhibitor of G9a activity, binding to its binding site and making it inaccessible. Several small molecule inhibitors have been developed with the ability to inhibit the catalytic activity of G9a and have been used in various in vitro and in vivo experiments (Park 2022). A study combining a G9a inhibitor (UNC0642 with an immunotherapy (anti-PD1) showed an increase in the effectiveness of immunotherapy, with greater survival and a lower incidence of acquired resistance to blocking the checkpoint inhibitor in murine melanoma (Kelly 2021). Studies have shown that inhibitors of G9a inhibit tumor growth by blocking cell cycle progression, triggering apoptosis, or inducing autophagic cell death (Liao 2023).

In 1914, Dexter described the Notch gene as being responsible for the notch phenotype on the wing margins of Drosophila melanogaster, and in the 1970s Spyros cloned and sequenced the Locus notch. Notch receptors are heterodimeric cell membrane proteins that have 5 Nocth ligands (DLL1, 2 and 3 and Jagged 1, 2), 4 of which have been identified in humans and rodents (Notch 1, 2, 3 and Notch 4).

Notch is known as a conserved signaling pathway in multicellular animals and is involved and acts in the regulation of cell cycle and proliferation, cell differentiation and fate, stem cell maintenance, survival, response to hypoxia, maintenance of homeostasis of adult tissue and apoptosis and in epitheliomesenchymal transitions (Oliveira Filho 2020).

It is known that positive signaling of the Notch1 pathway specifically contributes to the development of melanoma, allowing these cells to survive and proliferate in stressful, hypoxic environments (Ayaz 2014, Bedogni 2014, Tang 2019, Zhang 2016). This is because elevated Notch has been associated with antitumor immunity (Yang 2019), resistance to MEK inhibitors (Porcelli 2021), and MITF repression, leading to an invasive phenotype. Notch is involved in the progression of cutaneous melanoma, and that, from the interaction of melanoma cells with keratinocytes of the epidermis, there would be an activation of the Notch pathway, providing a phenotype capable of melanoma cells invading the basal membrane, passing to the vertical growth phase (Golan 2019). G9a stimulates the entire process of carcinogenesis through activation of notch1.

Research is focused on developing selective and specific KMT inhibitors. This is critical to minimizing unwanted side effects and maximizing therapeutic benefits. In addition to therapeutic applications, researchers are also investigating the molecular mechanisms by which KMT inhibitors affect chromatin structure, gene regulation, and cellular processes. This may lead to a deeper understanding of epigenetic regulatory systems, including the action of these drugs on other tissues and organs, understanding side effects.

KMT inhibitors can be classified into two main types: selective and broad-spectrum inhibitors. The former are designed to target a specific KMT enzyme or a subset of related KMTs that have similar targets. This allows for a more precise approach, targeting specific epigenetic modifications at specific

histone sites. Examples of selective inhibitors include those that target KMTs that act on H3K9 or H3K27 methylations. Broad-spectrum inhibitors: target multiple KMTs less specifically. They affect a broader spectrum of epigenetic modifications and may have more widespread effects on cells. This can be beneficial for manipulating gene expression generally, but it can also result in side effects. Importantly, as with any pharmacological development, safety, efficacy, and specificity are crucial considerations when developing KMT inhibitors for clinical applications.

2 CONCLUSIONS

Experimental studies with lysine histone methyltransferase (KMTs) inhibitors are ongoing in several fields of scientific research, including molecular biology, epigenetics, oncology and drug development. These studies aim to better understand the function of KMTs, investigate their roles in normal and pathological cellular processes, and evaluate the therapeutic potential of KMT inhibitors in various diseases. Clinical trials of lysine methyl transferase inhibitors in the treatment of advanced cutaneous melanoma are still in the early stages. They are being tested alone and in combination with immunotherapeutics (anti-CTLA4, anti-PD1) and targeted therapy agents (BRAF/MEK inhibitors). It is believed that these drugs can reverse resistance to immunotherapies and targeted therapy agents, making treatment more effective. It is also expected that they may affect genes involved in the metastasis of cutaneous melanoma, reducing the ability of melanoma to affect other organs in the body.

These studies on KMTs and their inhibitors are crucial for understanding the epigenetic mechanisms underlying diseases and biological processes, aiming to modulate the activity of these enzymes for targeted treatments of diseases related to epigenetic dysfunctions, such as cancer and genetic disorders. This places these experimental researches as a fundamental focus for the advancement of knowledge towards the clinical application of these compounds.

There are many challenges associated with the clinical use of KMTs inhibitors: specificity - these inhibitors must be selective for target KMTs and not negatively affect other biological pathways; side effects – as KMTs are involved in many physiological cellular processes, the possibility of side effects is great and should be studied and monitored; tumor heterogeneity – the heterogeneity of tumor cells makes it difficult to predict which patients will respond to KMT inhibitors; resistance – as with immunotherapy and targeted therapy treatments, tumor cells can develop resistance to KMT inhibitors over the course of their use. The development of new drugs is a long and complex process. Integration and cooperation among researchers is essential to speed up the discovery of new treatments for advanced cutaneous melanoma.

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