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Histone epigenetic modifications and their relationship with cancer: a comparative medicine view

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ABSTRACT: Epigenetic modifications have become highly important in the study of cancer pathogenesis due to research showing that changes in the expression of DNA-associated proteins can affect gene expression but may be reversible after treatment. The changing histones are being studied on a large scale in medicine while recent studies also show this relationship in veterinary medicine. Histone deacetylation is related to tumor progression and overexpression of histone deacetylases (HDACs) is responsible for these changes. The silencing of tumor suppressor genes related to epigenetic changes favors tumor progression; however, using HDAC inhibitors has been shown to effectively reverse these histone changes while having anticancer effects. This research provided an overview of comparative medicine between humans and dogs concerning epigenetic changes while showing the physiological mechanisms and the relationship between cancer and epigenetics, specifically regarding histone acetylation and deacetylation. This overview should contribute to a better understanding of epigenetics and cancer and their relationship with new target-molecular therapies in veterinary medicine and the importance of such studies.
Key words: hypoacetylation, canine, deacetylation, tumor, treatment.

Modificações epigenéticas de histonas e sua relação com o câncer: uma visão da medicina comparada

RESUMO: Mudanças epigenéticas assumiram importância na patogênese do câncer a partir de pesquisas que mostraram que mudanças na expressão de proteínas associadas ao DNA podem afetar a expressão gênica e podem ser reversíveis após o tratamento. As alterações nas histonas têm sido estudadas em larga escala na medicina, particularmente no câncer de mama, e estudos recentes mostram essa relação também na medicina veterinária. A desacetilação das histonas está relacionada à progressão tumoral e a superexpressão de histonas desacetilases (HDACs) é responsável por essas alterações. O silenciamento de genes supressores de tumor relacionados a alterações epigenéticas favorece a progressão tumoral, entretanto, o uso de inibidores de HDAC é eficaz em reverter as alterações nas histonas e tem efeitos anticâncer. Uma visão da medicina comparada entre humanos e cães em relação às alterações epigenéticas, será o objetivo deste trabalho, mostrando os mecanismos fisiológicos e a relação entre o câncer e a epigenética, especificamente com a acetilação e desacetilação de histonas. Essa visão contribuirá para um melhor entendimento da epigenética e do câncer, bem como a relação com as novas terapias moleculares-alvo na medicina veterinária e a importância dos estudos neste contexto.
Palavras-chave: hipoacetilação, canino, desacetilação, tumor, tratamento.

INTRODUCTION

Dogs have been identified and used as a good experimental model for studying several human diseases, including malignant neoplasms, making them an attractive model species for cancer research (LINDBLAD-TOH et al., 2005; RANIERI et al., 2013). The interdisciplinary field of comparative oncology offers a unique and powerful opportunity to learn more about the universal risk of developing cancer and its progression through epidemiologic, genetic and epigenetic investigations. Working across species, human and veterinary medicine researchers can combine scientific findings to understand more quickly the origins of cancer and translate those findings into new therapies to benefit humans and animals (SCHIFFMAN et al., 2015).

Studies point to epigenetic mechanisms in cancer development (WITTENBURG et al., 2011; FERRARESSO et al., 2014; ECKSCHLAGER et al., 2017). Epigenetics was defined as a discipline over 50 years ago and originally described changes in organism development that could not be explained by DNA mutations (HUANG et al., 2011). Epigenetic modifications are hereditary alterations in gene expression that do not change;however, the DNA

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sequence. Epigenetic patterns can be influenced by the environment so that phenotypic changes can be transmitted to offspring (FEINBERG, 2001).

In recent years, these changes have been clearly shown to affect cancer development (MOMPARLER, 2003; GUERRIERO et al., 2017) while it is also known that cancer development and progression can be potentially reversible with drug treatments, thus improving patient prognosis (KRISTENSEN et al., 2009).

Predicting the tumor response to a particular therapy should be a priority in cancer research (MARQUARD et al., 2008). Understanding all these epigenetic changes and their contribution to tumorigenesis is very important for definitive progress in diagnosis, prognosis and therapy of human and animal cancer, becoming; therefore, urgent to understand the functional genome, i.e., the changes defined by the regulatory mechanisms that cover the genetic structure (JOVANOVIC et al., 2010).

This review described the physiological epigenetic mechanisms related to histone acetylation and deacetylation, as well as the relationship between epigenetic alterations observed in human and canine tumors, looking to correlate these alterations with disease severity, evolution, and prognosis while highlighting the importance of comparative medicine.

DEVELOPMENT

Physiological factors in histone acetylation

The term epigenetics comes from the Greek prefix epi, which means „above or above something" (PRAY, 2004). Epigenetic changes are described as hereditary changes in the mechanisms controlling gene expression, transmitted by meiosis or mitosis, but which do not alter the nucleotide base sequences of the DNA molecule (EGGER et al., 2004; SANDOVAL & ESTELLER, 2012). Epigenetic patterns may undergo environmental influence in such a way that phenotypic changes can be transmitted to the descendants while also being physiological and reversible (FEINBERG et al., 2001).

Epigenetic mechanisms change the chromatin accessibility for transcriptional regulation, by modifying DNA or/and modifying or rearranging nucleosomes. These mechanisms are critical components for normal cell development and growth (MULLER & PRADO, 2008). As of today, the post-translational modifications of histones already identified included acetylation, phosphorylation, methylation, monoubiquitination, sumoylation, ADP ribosylation, deamination, propionylation and butyrylation (KOUZARIDES, 2007; SANDOVAL & ESTELLER, 2012; KEBEDE et al., 2015). The objective of this research is restricted to analyzing only histone acetylation and deacetylation mechanisms.

Unlike the genome, which is identical in different cell types, the epigenome is dynamic and varies from cell to cell. Some characteristics observed in epigenetic mechanisms differ from those observed in conventional genetics, such as reversibility, positioning effects, and the ability to act at unexpected distances, which may be greater than a single gene (FEINBERG et al., 2001; SANDOVAL & ESTELLER, 2012).

Reversibility gives chromatin dynamism while being controlled by enzymes that act oppositely by either adding or removing modifications. As an example, we cite histone acetyltransferase (HATs) and deacetylases (HDACs) that control histone acetylation as well as histone methyltransferases (HMTs) and demethylases (HDMs) that control histone methylation. Importantly, enzymes responsible for histone modifications can also act on non-histone proteins (Sandoval and Esteller, 2012); however, these implications will not be addressed in this review.

The two main mechanisms involved in epigenetics include changes in histones and DNA methylation patterns, as well as the changing structure of DNA covalent bonds (D´Alessio and Szyf, 2007). These modifications result in greater or lesser accessibility of the chromatin, which regulates the local or global transcription of genes based on DNA modifications and nucleosome rearrangements (LUND & LOHUIZEN, 2004). In addition to these main epigenetic mediators, the presence of non-coding RNAs can also interfere with gene transcription (TANG & HO, 2007).

In the nuclei of all eukaryotic cells, genomic DNA is highly folded, restricted and compacted by histone and non-histone proteins in a dynamic polymer called chromatin. Histones are the main DNA packaging proteins and were previously believed to have only this structural function; however, a more recent broader view has shown that these proteins play an important role in post-translational gene expression and are directly linked to changes in their amino-terminal structures. Histones are small basic proteins made up of a globular domain and a more flexible and charged amino-terminal (histone tail) that protrudes from the nucleosome (JENUWEIN & ALLIS, 2001).

Modifications in chromatin expression are directly linked to epigenetic changes. The

distinct levels of chromatin organization depend on the higher-order dynamic structure of the nucleosomes consisting of 146 base pairs of DNA, which represent the basic repeat unit of chromatin. In each nucleosome, approximately two superhelical turns of DNA involve an octamer of essential histone proteins formed by four histones: a tetramer of H3 and H4 and two dimers of H2A and H2B (LUGER et al., 1997). Histones are responsible for the organization and union of the nucleosome. Also, the H1 histone is associated with DNA and contributes to its compaction; however, it is not yet clear how the nucleosomal matrices that contain the ligand histone (H1) twist and fold this chromatin fiber into increasingly compacted filaments, leading to defined higher-order structures (Figure 1) (MULLER & PRADO, 2008).

H3 and H4 histones are subjected to post-translational modification, thus, the modification of the lysine N-terminal group in histones by acetylation or deacetylation alters the configuration of nucleosomes. The positive charge on non-acetylated lysines in histones is attracted to the negatively charged DNA producing a condensed chromatin state that inhibits gene transcription. Conversely, the acetylation of lysines removes its positive charge and results in an open chromatin structure, which facilitates gene transcription (ELSHEIKH et al., 2009; CHUN et al., 2017).

The modifications of histone tails include acetylation, methylation, phosphorylation, and ribosylation, among others, each of which can significantly affect gene expression. The most studied histone modifications are acetylation and deacetylation, as well as methylation and demethylation (JENUWEIN & ALLIS, 2001; XAVIER et al., 2020). Specific changes in histone tails can lead to either silencing or expressing the genes that can favor or inhibit the appearance of cancer. Still, these modifications can be isolated or combined and this is already well documented in medicine and gave rise to the histone code (PRAKASH & FOURNIER, 2017).

Histone acetylation is controlled by two enzymes: Histone acetyltransferases (HAT) and deacetylases (HDAC). The HDAC family is divided into zinc-dependent enzymes (classes I, IIa, IIb and IV, of which there are 11 enzyme subtypes) and independent zinc enzymes (class III, also called sirtuins), which require NAD + for their catalysts (CHEUNG et al., 2000; SETO & YOSHIDA, 2014). HDACs 1 and 2 comprise class I enzymes, while HDAC 6 belongs to the class IIa family (CHEN et al., 2015).

HAT enzymes are responsible for adding the acetyl group to the amino-terminal region of histones, more specifically in lysines (K). HDACs in turn remove the acetyl group located on the lysine amino-terminal; however, these enzymes do not act specifically on certain histones or amino-terminal regions, they can act on a variety of proteins, including non-histone proteins (BARNEDA-ZAHONERO & PARRA, 2012; DELL'AVERSANA et al., 2012; PRAKASH & FOURNIER, 2017).

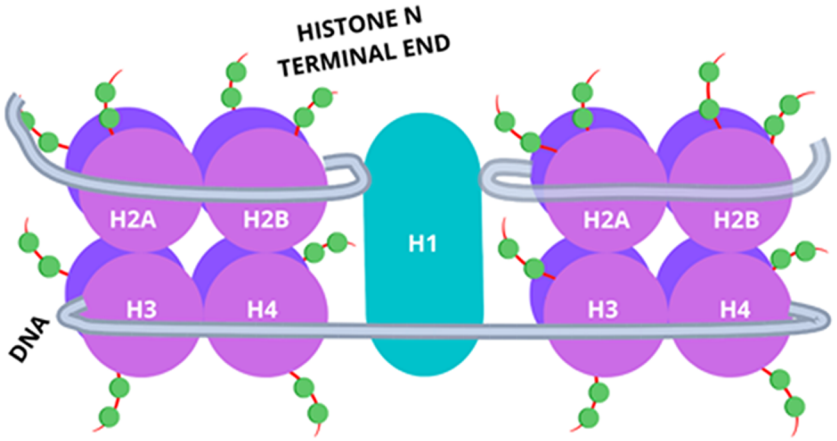

Figure 1 - Nucleosomal organization of DNA. The DNA strand is coiled around an octamer of histone subunits and the binding DNA is stabilized by histone H1.

The balance between histone acetylation and deacetylation is essential for regulating cell proliferation. The imbalance between histone acetylation and deacetylation is associated with the silencing of tumor suppressor genes, which has allowed a better understanding of carcinogens and cancer progression (Figure 2) (MENDITI & KANG, 2007). The abnormal increase in HDAC activity can lead to the inactivation of transcription of tumor suppressor genes, inhibiting its transcription due to the deacetylation of histones followed by DNA methylation, inactivating the gene (D'ALESSIO & SZYF, 2006).

Histone hyperacetylation leads to apoptosis of tumor cells, cell differentiation, interruption of the cell cycle and inhibition of tumor angiogenesis. This knowledge has been used to improve the development and application of HDAC inhibitors (iHDAC) as anticancer agents, including their use combined with other anti-cancer drugs, leading to improved prognosis in cancer patients (BOLDEN et al, 2006).

Epigenetics and cancer

Epigenetic events, including histone modifications, are crucial to establishing the correct programming of gene expression while errors in these processes can lead to aberrant gene expression and the loss of anticancer checkpoints (Strahl and Allis, 2000). Cancer is a process in which genetic and epigenetic errors accumulate and transform a normal cell into invasive cells or metastatic tumor cells (RODENHISER & MANN, 2006).

In human medicine, some research on this topic has already been carried out and promising results have been evidenced, showing the importance of epigenetic changes in carcinogenesis (Table 1). A study conducted on human patients with cutaneous lymphoma showed high expression of HDACs 2 in the most aggressive tumor types compared to the indolent types (MARQUARD et al., 2008). Also in humans, several FDA-approved iHDACs are being used specifically to treat patients with refractory cutaneous lymphomas, pretreated with chemotherapy, increasing patient survival time (LANSIGAN & FOSS, 2010).

The histone acetylation pattern was also assessed in breast tumors of women. ELSHEIKH et al. (2009) evaluated the histone acetylation pattern in breast tumors of 880 women using immunohistochemistry and observed high and low levels of global acetylation in tumors with better and worse prognoses, respectively. They concluded that changes in histone acetylation, in different breast cancer carcinoma subtypes, may

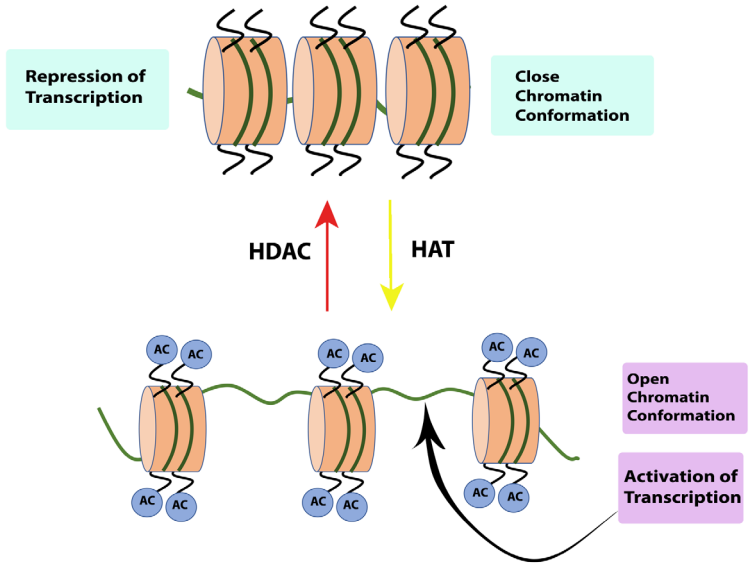

Figure 2 - Histone acetylation is controlled by histone acetyltransferase (HAT) enzymes that add acetyl groups in the N-terminal region of histones, activating gene transcription by favoring chromatin opening. In turn, histone deacetylases (HDAC) enzymes are responsible for the N-terminal deacetylation of histones, compacting chromatin and repressing gene transcription.

Table 1 - Studies related to epigenetic modifications in histones and deacetylase enzymes in human and animal cancers.

Reference	Species	Cancer	Main results
MARQUARD et al., 2008	Human	Cutaneous lymphoma	High expression of HDAC2.
ELSHEIKH et al. 2009	Human	Breast cancer	Low levels of acetylation of H4K16Ac. High levels of acetylation of H3K18Ac.
SUZUKI et al., 2009	Human	Breast cancer	Hypoacetylation of H4Ac and H4K12Ac in carcinomas in situ and invasive carcinomas.
KRUSCHE et al., 2005	Human	Breast cancer	High HDAC1 expressions correlate with smaller tumors and longer survival time.
MULLER et al., 2013	Human	Breast cancer	High expression of HDAC2 and HDAC3 in more aggressive tumors and patients who had shorter survival times.
TOH et al., 2004	Human	Esophageal squamous cell carcinomas	Hypoacetylation of histone H4Ac correlated with neoplasms in the advanced evolution stage and an increase in acetylation with a better prognosis.
FUJIWARA-IGARASHI et al., 2016	Canine	Immunophenotype B canine lymphoma cell lines	Low acetylation of H3 correlated with low expression of P16 mRNA.
ETO et al., 2019	Canine	Urothelial carcinomas	Low expression of H3K9Ac in urothelial carcinomas and correlation with poor prognosis.
SIERRA, 2019	Canine	Cutaneous lymphoma	Hypoacetylation of H3K9 and aberrant expression of H4K12Ac associated with HDAC2 related to shorter survival times.
SENHORELLO, 2020	Canine	Mammary tumors	Lower acetylation of H3K9Ac in breast carcinomas, expression of HDAC1 and HDCA2 lower in neoplastic breast tissues and HDAC6 more expressed in neoplastic breast tissues.
SENA et al., 2022	Canine	Soft tissue sarcomas	High correlation between the expression of H3K9Ac and the number of mitotic figures and intense immunolabelling of HDAC1.

be related to the silencing of tumor suppressor genes, especially in those with low levels of acetylation with clinical implications, since the use of iHDACs can reverse hypoacetylation and allow previously silenced genes to be expressed.

Suzuki et al. (2009) also studied the expression of HDACs and the relationship with histone acetylation in breast tumors of women, noting that more aggressive tumors showed hypoacetylation with high expression of HDACs, thus justifying using HDAC inhibitors early to treat patients with tumors exhibiting these characteristics.

In breast tumors of women, evaluation of the expression of HDAC1 and HDAC3 showed that high HDAC1 expression is correlated with a better prognosis. Despite proving to be an independent prognostic marker since it directly correlates with a longer survival time for patients, the HDAC1 expression did not correlate with classic prognostic markers, thus showing the potential to become a prognostic marker for deciding whether or not to pursue treatment (KRUSCHE et al., 2005).

Conversely, MULLER et al. (2013) observed a high expression of HDAC isoenzymes 2 and 3 in more aggressive breast tumors in humans and patients who had shorter survival times. The fact that hyperacetylation using HDAC inhibitors favors apoptosis, transforms the tumor microenvironment and reduces tumor size has led to clinical studies in women with breast tumors, already in phase III (MULLER et al., 2013; GUERRIERO et al., 2017).

In other neoplasms such as squamous cell carcinoma of the esophagus in humans, the hypoacetylation of histones H3 and H4 has been correlated with neoplasms in the advanced evolution stage and increase in acetylation with better prognosis (TOH et al., 2004).

In vitro tests have shown effective action of iHDACs in breast tumor cell lines, as cell proliferation was inhibited in all cell lines treated with trichostatin A, an inhibitor of histone deacetylases, which led to H4 hyperacetylation. In the same study, the in vivo action of iHDAC in rat breast carcinoma models was evaluated. A high occurrence of benign tumors was observed in treated animals compared to untreated animals. The authors suggested that iHDACs can be potentially used for anticancer therapy in breast tumors (VIGUSHIN et al., 2001; AUDIA & CAMPBELL, 2016).

The expression of p16, a tumor suppressor gene, was evaluated *in vitro* in immunophenotype B canine lymphoma cell lines. In this study, this protein low expression was correlated with H3 hypoacetylation in tumor cells. Once treated with iHDACs TSA, the cells not only became hyperacetylated but started to express a greater amount of p16, suggesting a relationship between epigenetic alteration and gene silencing that is potentially reversible with drug treatment (FUJIWARA-IGARASHI et al., 2016).

Based on *in vitro* and *in vivo* experimentation using animal models, some authors reported that histone hyperacetylation results in apoptosis of tumor cells, due to the expression of tumor suppressor genes. Using canine tumor cell lines treated with iHDACs, researchers have shown increased histone acetylation in some tumors, especially in T lymphoma, mastocytoma, osteosarcoma and histiocytic sarcoma strains. These studies add that, in addition to hyperacetylation, apoptosis and cytotoxicity were also induced (KISSEBERTH et al., 2008). More recently, other researchers have evidenced changes in the tumor microenvironment that favor the immune system (ELSHEIKH et al. 2009; AUDIA & CAMPBELL, 2016; MOUFARRIJ et al., 2020).

Studies evaluating the influence of iHDACs on canine tumor lines have also shown encouraging results (ELSHAFAE et al., 2017; MURAHARI et al., 2017). Strains of canine prostate tumors, a study model for prostate tumors in humans, were evaluated for their influence on proliferation, migration and metastatic potential when subjected to treatment with iHDACs. Results showed decreasing cell proliferation, differentiation and *in vitro* migration depending on the dose used. Additionally, in animal models treated with iHDACs, a decrease in the metastatic potential of these cells was observed (ELSHAFAE et al., 2017).

A similar study evaluating osteosarcoma cell lines treated with iHDACs AR-2 and SAHA, combined or not with doxorubicin, induced apoptosis of tumor cells, mainly with the use of AR-2 and the combination of AR- 2 and doxorubicin, leading to the conclusion that these therapies could be promising for treating this disease in dogs (MURAHARI et al., 2017).

ETO et al. (2019) evaluated the H3K9 acetylation pattern of urothelial carcinomas in dogs and observed lower acetylation compared to normal tissue. Additionally, these changes had an impact on the prognosis, culminating in a shorter disease-free time after surgery. In that same study, strains of urothelial carcinoma treated with HDAC inhibitors exhibited an anti-cancer therapeutic response.

Three studies by our research group showed important epigenetic changes in histones in canine neoplastic tissues. In cutaneous lymphoma in dogs, in addition to hypoacetylation of H3K9 compared to normal lymph nodes, aberrant expression of H4K12Ac associated with HDAC2 was found to be related to shorter survival times in patients (SIERAA, 2019). The study of mammary tumors in bitches showed lower acetylation of H3K9Ac in breast carcinomas compared to normal breast tissue, in addition, the expression of HDAC1 and HDCA2 was lower in neoplastic breast tissues compared to normal tissue. In contrast, HDAC6 was more expressed in neoplastic breast tissues. Furthermore, low HDAC1 expression was associated with the presence of lymph node metastasis, behaving as a prognostic factor (SENHORELLO, 2020). The evaluated soft tissue sarcomas (STSs) showed a high correlation between the H3K9Ac expression and the number of mitotic figures, while immunolabelling of HDAC1 was intense compared to the expression of HDAC2 and HDAC6 (SENA et al., 2022).

CONCLUSION

In both medicine and veterinary medicine, epigenetic modifications represent an important step in the pathogenesis of tumors and knowledge of such changes may determine specific target therapies and predict response to treatment. Epigenetic changes in histone acetylation and expression of HDACs are potentially reversible with the use of iHDACs, indicating a promising therapy to treat cancer in humans and animals; however, further clinical studies are still needed to prove the use of these therapies in Veterinary Medicine.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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