ARTIGO ORIGINAL

ASSOCIATION BETWEEN LINE-1 HYPOMETHYLATION IN CANINE MAMMARY TUMOR TISSUES: IMPLICATIONS FOR DIAGNOSIS AND RESEARCH

Associação entre hipometilação de LINE-1 em tecidos tumorais mamários caninos: implicações para diagnóstico e pesquisa

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ABSTRACT

With the increase in dog life expectancy, canine mammary cancer has become the most critical and frequent neoplasm in dogs. As in humans, this type of cancer has spontaneous development and is influenced by several risk factors, such as age and hormonal exposure in addition to genetic and epigenetic factors. Thus, knowing that epigenetic patterns influence a variety of biological mechanisms, such development cancer, this study aimed to correlate the methylation pattern of LINE-1 elements in healthy and neoplastic canine mammary tissue, as well as in isolated cell lines of carcinoma canine with tumoral aggressiveness degree. To this end, a retrospective study was conducted in which genomic DNA was extracted from histological sections of biopsies and cell lines using the phenol/chloroform method. To determine the percentages, the genomic DNA was pretreated with methylation-sensitive endonucleases and then amplified by qPCR with primers specific for the L1td1 gene. The results revealed that both the tumor cell lines and the canine adenoma and inflammatory carcinoma samples are hypomethylated relative to normal tissue. However, it was not possible to confirm the relationship between the percentage of LINE-1 hypomethylation and tumor aggressiveness in canine breast cancer. Therefore, the combined analysis of our results allows us to conclude that the methylation pattern of LINE-1 elements can be used as a molecular biomarker to differentiate normal canine mammary tissue from neoplastic canine mammary tissue. However, to confirm whether there is a relationship between the degree of methylation of LINE-1 elements and tumor staging, further studies with canine samples are needed.

Keywords: Epigenetics, canine mammary cancer, LINE-1, inflammatory carcinoma, adenoma, neoplastic cell culture canine.

RESUMO

Com o aumento da expectativa de vida canina, o câncer de mama canino tornou-se a neoplasia mais crítica e frequente em cães. Assim como em humanos, esse tipo de câncer tem desenvolvimento espontâneo e é influenciado por diversos fatores de risco, como idade e exposição hormonal, além de fatores genéticos e epigenéticos. Assim, sabendo que padrões epigenéticos influenciam uma variedade de mecanismos biológicos, como o desenvolvimento do câncer, este estudo teve como objetivo correlacionar o padrão de metilação dos elementos LINE-1 em tecido mamário canino saudável e neoplásico, bem como em linhagens celulares isoladas de carcinoma canino com o grau de agressividade tumoral. Para tanto, foi realizado um estudo retrospectivo no qual o DNA genômico foi extraído de cortes histológicos de biópsias e linhagens celulares utilizando o método fenol/clorofórmio. Para determinar as porcentagens, o DNA genômico foi pré-tratado com endonucleases sensíveis à metilação e então amplificado por qPCR com primers específicos para o gene L1td1. Os resultados revelaram que tanto as linhagens celulares tumorais quanto as amostras de adenoma e carcinoma inflamatório canino são hipometiladas em relação ao tecido normal. No entanto, não foi possível confirmar a relação entre a porcentagem de hipometilação do LINE-1 e a agressividade tumoral no câncer de mama canino. Portanto, a análise combinada de nossos resultados nos permite concluir que o padrão de metilação dos elementos do LINE-1 pode ser usado como um biomarcador molecular para diferenciar o tecido mamário canino normal do tecido mamário canino neoplásico. No entanto, para confirmar se existe uma relação entre o grau de metilação dos elementos do LINE-1 e o estadiamento tumoral, estudos adicionais com amostras caninas são necessários.

Palavras-chave: Epigenética, câncer mamário canino, LINE-1, carcinoma inflamatório, adenoma, cultura de células neoplásicas caninas.

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INTRODUCTION

Canine mammary tumors (CMTs) are a highly heterogeneous group of neoplasms that represent a serious clinical problem in veterinary medicine[1]. Of unknown etiology, they represent approximately 25 to 70% of all cancers affecting intact female dogs. Its prevalence may vary depending on geographic location. Various risk factors have been associated with its development, with hormonal, nutritional, and genetic factors being the most prominent, with sex hormones being one of the main risk factors for its development[2,3]. Histologically, CMTs can be classified as benign or malignant epithelial tumors, with approximately more than 50% of CMTs classified are histologically malignant. Of this group, the most common tumor type is tubular carcinoma (adenocarcinoma), followed by papillary, solid, and complex carcinomas, as well as carcinosarcoma[4,5].

In general, the malignant CMTs present with typical characteristics, such as an irregular chromatin pattern, destructive growth, discontinuous basement membranes, vascular invasion, necrosis, a high mitotic index, and cellular polymorphism. In contrast, benign breast tumors are often encapsulated and do not exhibit invasive growth[6,7]. Even though it represents one of the most important types of neoplasia in dogs, the pathogenic mechanisms molecular underlying the formation and development of canine mammary tumors have not yet been fully understood[8]. In this regard, genetic alterations and molecular signatures underlying their development in canine mammary cancers have already been studied[9,10] with few studies addressing global DNA methylation changes[11,12].

Even though DNA methylation is a mechanism independent of hereditary patterns and/or genetic mutations, its involvement is critical for the initiation and progression of neoplastic processes[13]. In the mammalian genome, methylation occurs at cytosine bases located at the 5' end of guanine bases, promoting the formation of CpG dinucleotide islands. These CpG dinucleotide islands play a central role in transcriptional repression (when most CpG dinucleotides on an island are methylated) or in transcriptional activation by hypomethylation of CpG dinucleotides. Thus, methylation and demethylation are central to transcriptional regulation, especially in tumor transformation processes[14,15,16].

In tumors, global loss of genome methylation (hypomethylation) is observed, and it is suggested that hypomethylation initiates and propagates carcinogenesis through the induction of chromosomal instability and transcriptional activation of oncogenes[17]. The relationship between epigenetic changes and tumor development is supported mainly by the occurrence of initial hypomethylation events that result in the transcriptional activation of oncogenes and, in a second moment, the transcriptional repression of tumor suppressor genes mediated by hypermethylation events[18].

Although hypermethylation of specific genes has been well characterized in cancer, the impact of global DNA hypomethylation still requires further study. Hypomethylation occurs mainly in repetitive elements and is primarily associated with genomic and chromosomal instabilities. It occurs in about 50% of breast cancers and correlates with histological grade, clinical stage, and malignancy[19].

Repetitive DNA elements are dispersed throughout the mammalian genome and constitute up to 75% of the human genome[20]. Among the different sequences, DNA class I transposable elements (LINE-1) belong to the long and interspersed L1 nuclear elements, which comprise around 34% of the canine genome[21]. The LINE-1 elements are a type of repetitive sequence dispersed throughout the genome. The study of their methylation pattern is widely used in understanding numerous multifactorial diseases, directly correlating to the global methylation of the genome[22]. During the development of the carcinogenic process, its hypomethylation is directly associated with genetic changes such as genomic instability, mutation of tumor suppressor genes, and alternative transcription of oncogenes[23,24]. Although numerous studies have investigated genetic alterations and underlying molecular signatures in canine mammary tumors, few studies have investigated epigenetic modifications in these elements[15]. In this sense, the hypomethylation of this region was demonstrated by Lee et al., who showed that primary canine mammary tumor cells and Circulating cell-free DNA (cfDNA) obtained from liquid biopsies were hypomethylated and had a high similarity with the results in humans. Thus, this study aimed to correlate the methylation pattern of LINE-1 elements in healthy and neoplastic canine mammary tissue, and to correlate their methylation status with the degree of aggressiveness of the CMTs.

MATERIALS AND METHODS

Statement ethical

To carry out the morphological and molecular study, canine non-neoplastic and neoplastic mammary tissue were selected from the archives of the Veterinary Pathology Laboratory at Universidade Paulista (UNIP), including healthy tissue and benign and malignant mammary neoplasms. For this reason, this study did not require prior approval from the Animal Research Ethics Committee, and was only authorized to be carried out with the approval of the person responsible for the tumor bank.

Biological material and sample selection

Samples previously fixed in 10% formaldehyde and embedded in paraffin were used to acquire histological sections five µm (micrometers) thick and subsequently stained with hematoxylin-eosin for evaluation under an optical microscope. Canine mammary histopathological samples were reevaluated and reclassified following the classification proposed by Goldschmidt et al., 2011[25]. A total of 15 specimens: 5 healthy tissues, five mammary adenomas, and five inflammatory breast carcinomas were selected.

To avoid interobserver variability in histological interpretation, all biopsies were reviewed by a single veterinary pathologist.

Cell Culture

The CM1 lineages, isolated from solid carcinoma, and MM4, isolated from a metastatic lymph node established by the Laboratory of Immunopathology and Cancer Biology (LaCIB), were kindly donated by Prof. Dr. Carlos E. Fonseca Alves. Both strains were cultivated in RPMI 1640 medium (Roswell Park Memorial Institute), which was supplemented with 10% of fetal bovine serum (FBS) and 1% antibiotics (100 μg/mL of streptomycin and 100 IU/mL of penicillin) in a humid oven at 37oC containing 5% CO2, with periodic repeats every 72h.[17].

Epigenetic Analysis

To carry out the methylation study of the LINE-1 promoter region, genomic DNA was extracted from histological sections of paraffin samples and canine mammary tumor cell cultures.

Deparaffinization

As an initial step, histological sections were incubated for 30 minutes at 57°C with 1 mL of xylene. Then, the samples were centrifuged at 12.000 RPM (Eppendorf/5804 R centrifuge) for 5 minutes, the supernatant discarded, and the precipitate (deparaffinized tissue samples) stored at -20°C for subsequent DNA extraction.

DNA extraction

To obtain genomic DNA (gDNA) from canine breast cancer tissues obtained by deparaffinization of histological sections, the precipitates were homogenized in extraction buffer (10 mM Tris, pH 3.0; 0.5% SDS, five mM EDTA) and digested with proteinase K (20 mg/mL) for 16h at 56°C. The following day, DNA was isolated by the phenol/chloroform/ alcohol method isoamyl. 18 The quantity and purity of gDNA extracted will be estimated in a spectrophotometry device, using the ratios OD 260/280 (≥ 1.8) and OD 260/230 (≥ 1.0) (NanoDrop 2000, Thermo Scientific, Uniscience).

DNA treatment

Each gDNA sample was divided into three tubes containing the same concentration (400 ng) and digested with MspI endonucleases (New England Biolabs, Beverly, MA, USA), which recognizes and cuts all CCGG sequences (methylated and unmethylated) and by HpaII (New England Biolabs, Beverly, MA, USA) which recognizes all CCGG sequences with the difference that Methylated CCGG sequences (5-meC) promotes the inhibition of its activity catalytic. In the third tube, the same amount of H2O (gDNA undigested = 100% control). All reactions were carried out separately with a final volume of 25 μL at 37°C for 16 hours and inactivated at 65°C for 15 minutes. After completion of enzymatic digestion, the samples were stored in a -20°C freezer.

Methylation-sensitive restriction enzyme digestion (MSRED) and qPCR

The methylation (5-meC) pattern of the region promoter of LINE-1 elements was determined by qPCR reactions containing SYBR

Green I Master 2x (5 µL) 0.5 µM specific initiators [(F: 3' GTC TTT GGG GAG TGA TGG AA 5') and (R: 3' TAA CAA TGA AGG CGA TGC AG 5')], 1.5 µL (25 ng) of treated gDNA (in the three conditions: H2O, MspI and HpaII), and 3 µL of nuclease-free H2O.

Calculations and Statistics

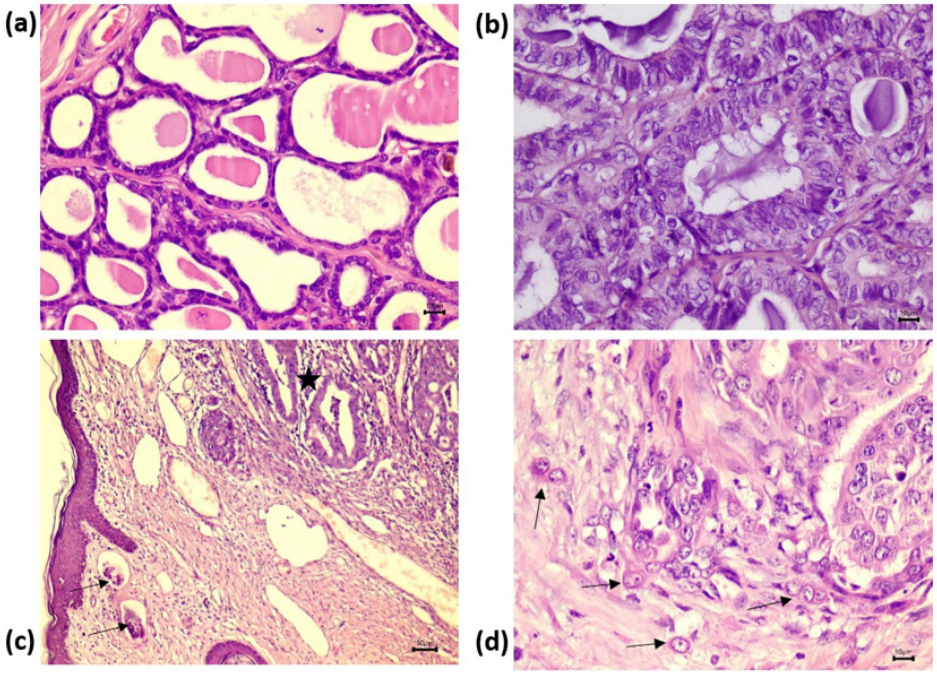
To obtain percentage values regarding methylation of samples, we used the formula [5-meC = (HpaIICt - MspICt) / CrtCt X 100] according to Nestor and collaborators,2012, with minor modifications[28]. A one-way ANOVA test determined the significance of the methylated LINE-1 level in the plasma. The ROC value, sensitivity, and specificity were calculated by GraphPad PRISM 7 software (GraphPad Software, La Jolla, CA).

RESULTS

Histopathological characterization

Histopathological analysis shows detailed morphological aspects related to intact breast tissue (**Fig. 1a**) and the versions neoplastic, respectively, benign, with an increase in local cellularity associated with discrete pleomorphism (**Fig. 1b**), and malignant, with carcinoma inflammatory pattern assuming a tubular pattern (star) and neoplastic embolization in lymphatic vessels (arrows) (**Fig. 1c**) and malignant cell invasion individually in adjacent interstitial sites (**Fig. 1d**).

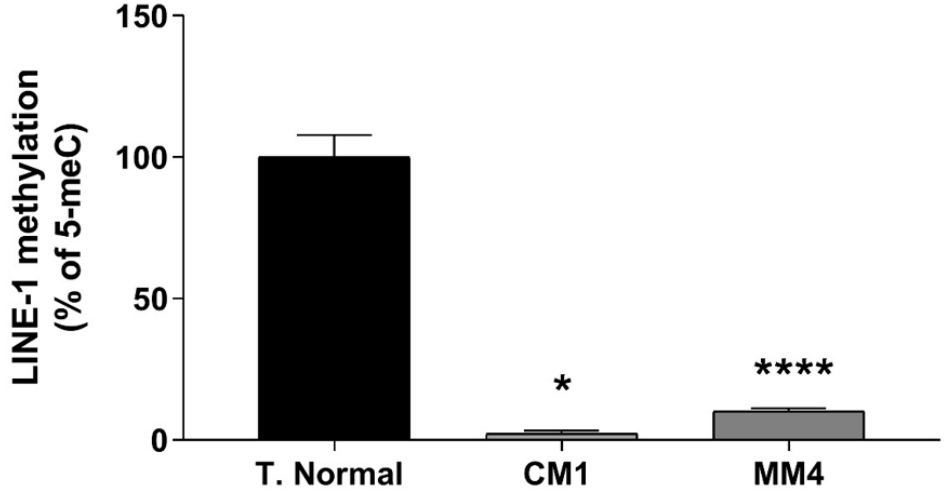
Figure 1: Histopathological classification of canine mammary tissue: Representative photomicrograph of normal mammary tissue (**a**), mammary tubular adenoma (**b**), and inflammatory mammary carcinoma with emphasis on the presence of neoplastic emboli in lymphatic vessels in the superficial dermis (arrows). Aggregate of neoplastic tubules (star)(**c**) and tubular growth, with free invasive neoplastic cells being observed in the dermis (arrows) (**d**). Images provided by Prof. Dr. José Guilherme Xavier.



Differential methylation of the LINE-1 between normal and mammary tumor cells canine

After standardizing the ideal cycling conditions for the set of specific primers for the promoter region of the L1td1 gene (LINE-1), the methylation status of the promoter region of the LINE-1 elements of the lineages' tumor and biopsy samples was determined by qPCR. The results regarding the methylation pattern of tumor lines presented in **Fig. 2** show that the promoter region of the elements LINE-1 of both canine breast tumor lines is hypomethylated relative to normal breast tissue. Interestingly, when we evaluated the methylation percentages only between tumor lineages, it was possible to note that the lineage isolated from the canine solid breast tumor, CM1, presented a lower methylation rate than the metastatic lineage, MM4.

Figure 2: Determination of the methylation status of the promoter region of the L1TD1 gene (Line-1) mammary tumor cells canine. The methylation status of the promoter region (5-meC) was investigated by qPCR analysis with specific primers after previous enzymatic digestion of the genomic DNA with the endonucleases MspI and HpaII. Relative methylation levels were determined by qPCR using the cycle threshold (Ct) method. Methylation values (5-meC) were obtained by calculating Ct $^{[HpaII]}$ - Ct $^{[MspI]}$ / Ct $^{[SE]}$. The results were represented as mean ± standard deviation of 5 independent biopsies performed in technical duplicates, normalized as a function of the control (100%).



Reduction in methylation of the LINE-1 between canine breast tumor samples

Regarding the characterization of the methylation pattern of the region promoter of LINE-1 elements from canine breast tumor samples, results similar to those obtained for the strains were observed in tumors. The tubular adenoma samples and the inflammatory carcinoma have a lower percentage of methylation in the region promoter of LINE-1 elements compared to the percentage observed in normal tissue. Additionally, the comparison between

the percentages of methylation of tumors alone revealed that tubular adenoma samples showed a lower percentage of methylation when compared to the percentage of methylation in Inflammatory carcinomas **(Fig. 3a)**. The results presented in **Fig. 3b** refer to the graphical comparison of the methylation percentages obtained in the samples evaluated. Our results show that both canine tumor lines, CM1 and MM4, are hypomethylated in tumor biopsies and that, corroborating the literature, both malignant cells and tumor tissue present significant hypomethylation of the promoter region of LINE-1 elements compared to the pattern observed in healthy breast tissue.

Figure 3: Determination of the methylation status of the promoter region of the *L1td1* gene (Line-1). The methylation status of the promoter region (5-meC) was investigated in canine breast tumor (**a**) by qPCR analysis with specific primers after previous enzymatic digestion of the genomic DNA with the endonucleases MspI and HpaII. (**b**) Graphical representation of the results obtained for LINE-1 methylation between cell lines and tumor tissues. Relative methylation levels were determined by qPCR using the cycle threshold (Ct) method. Methylation values (5-meC) were obtained by calculating $Ct^{[HpaII]} - Ct^{[MspI]} / Ct^{[SE]}$. The results were represented as mean ± standard deviation of 5 independent samples performed in technical duplicates, normalized as a function of the control (100%).

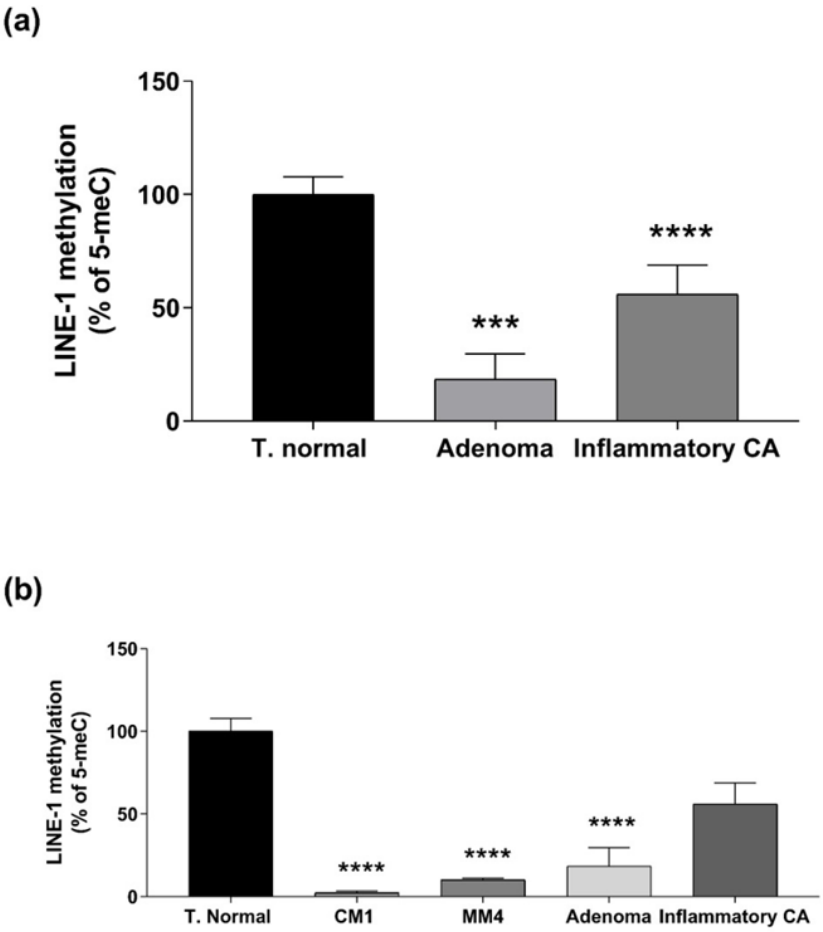


Table 1. Summary of tumoral samples with age, breed, and histological classification.

Sample	Age(year)	Breed	Histological classification
1	6	shih-tzu	adenoma
2	7	lhasa	adenoma
3	13	mongrel	adenoma
4	9	mongrel	adenoma
5	10	maltese	adenoma
6	14	shih-tzu	inflammatory carcinoma
7	13	cocker spaniel	inflammatory carcinoma
8	7	mongrel	inflammatory carcinoma
9	10	lhasa	inflammatory carcinoma
10	8	rottweiler	inflammatory carcinoma

DISCUSSION

Even with the significant improvements in human cancer diagnosis and treatment driven by the growing scientific advances in tumor biology achieved in recent years, these benefits have not yet been fully available in veterinary care, especially concerning canine breast tumors[29,30]. In this study, we retrospectively evaluated the LINE-1 methylation pattern in healthy breast tissue and in neoplastic conditions, associated with localized growth (adenoma) or with potential for distant growth (inflammatory carcinoma).

We found that both MTCs and tumor cell lines have a lower percentage of methylation in the LINE-1 region compared to the methylation pattern of healthy tissue, consistent with studies in humans and dogs that demonstrated the relationship between LINE-1 hypomethylation and tumor development[31]. As in humans, canine neoplasia is considered a disease of multifactorial etiology associated with genetic, environmental, and epigenetic factors. We know that benign and malignant neoplasms differ clinically by presenting distinct growth characteristics, degree of differentiation, and metastatic potential[32].

Its hypomethylation, in addition to being related to genomic instability events, causes transcriptional activation that will result in retrotransposition of the transposable element and chromosomal alteration[33,34]. Furthermore, these sequences, due to their high frequency in the genome, constitute approximately 34% of the canine genome[21]; LINE-1, can be used as surrogate markers for global methylation status. In normal cells, CpG sites within LINE-1 are generally methylated, thus maintaining transcriptional inactivation and inhibition of retrotransposition, as observed in our results in normal tissue[35].

Epigenetically, in humans, loss of methylation of LINE-1 regions of cancer cells is directly linked to increased expression of chimeric L1 transcripts, which have been widely used as an epigenetic marker of malignancy[36]. Unlike results obtained in human clinical studies, this relationship was not confirmed for the canine samples in this study. Comparative analysis of the LINE-1 methylation percentage between tumor tissues and tumor cell lines revealed that the tumor classified as benign and the non-metastatic

cell line, both less aggressive, presented a lower methylation percentage (hypomethylation) when compared to the percentage value of the tumor classified as malignant and the metastatic cell line.

These results contradict the literature on human mammary neoplasms, since tubular adenomas, characteristically characterized by localized growth and low aggressiveness, were hypomethylated compared to inflammatory carcinomas, which typically present rapid growth, intense invasiveness, potential for dissemination, and poor prognosis. However, they are in line with observations demonstrated in a study that evaluated 56 dogs. Enrolling healthy controls, dogs diagnosed with mammary tumors (benign and malignant) and other types of tumors showed hypomethylation of LINE-1 in liquid biopsies, and, similarly to our findings, the levels of LINE-1 hypomethylation were even higher in benign tumors[37]. These results contrast with evidence from studies in human cancer, such as colorectal and hepatocellular carcinomas, showing an increase in hypomethylation associated with a worse prognosis, characterized by an increased tumoral recurrence and shorter overall survival [38,39,40]. Also, LINE-1 hypomethylation is more pronounced in liver metastases compared to matched primary tumors[41,42].

Still, in a study with gastric carcinomas, Min and collaborators evidenced that LINE-1 methylation is reduced in lesions with lymphatic and venous invasion, irrespective of the tissue-storage type[43]. As previously mentioned, one of the characteristics of inflammatory carcinoma is tumoral embolization, mainly in lymphatic vessels; however, the LINE-1 methylation rate was higher than that of mammary adenomas. On some occasions, the level of methylation varies according to the site of the tumor, such as in neuroendocrine tumors (NETs). LINE-1 hypomethylation predicts tumor progression in small intestine/colorectal and pancreatic NETs but not in gastric and appendiceal NETs[44].

CONCLUSION

The methylation pattern of LINE-1 elements can be used as a molecular biomarker to differentiate normal canine mammary tissue from neoplastic canine mammary tissue. However, to confirm whether there is a relationship between the degree of methylation of LINE-1 elements and tumor staging, further studies with canine samples are needed.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authorship Statement

RAFS: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. PASCJ: Acquisition, analysis, and interpretation of data, Formal analysis, Investigation, Data curation, and Writing - original draft. MAL: Investigation, Software, Validation; JGX: Investigation, Methodology, Writing – review & editing.

Data Availability

All datasets generated for this study are included in the manuscript.

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REFERENCES

1. Valdivia G, Alonso-Diez Á, Pérez-Alenza D, Peña L. From Conventional to Precision Therapy in Canine Mammary Cancer: A Comprehensive Review. Front Vet Sci. 2021 Feb 17;8.
2.Valdivia G, Alonso-Diez Á, Pérez-Alenza D, Peña L. From Conventional to Precision Therapy in Canine Mammary Cancer: A Comprehensive Review. Front Vet Sci. 2021 Feb 17;8.
3.Vazquez E, Lipovka Y, Cervantes-Arias A, Garibay-Escobar A, Haby MM, Queiroga FL, et al. Canine Mammary Cancer: State of the Art and Future Perspectives. Animals. 2023 Oct 9;13(19):3147.
4.Salas Y, Márquez A, Diaz D, Romero L. Epidemiological Study of Mammary Tumors in Female Dogs Diagnosed during the Period 2002-2012: A Growing Animal Health Problem. PLoS One. 2015 May 18;10(5):e0127381.
5.Nakagaki KYR, Nunes MM, Garcia AP V, Nunes FC, Schmitt F, Cassali GD. Solid Carcinoma of the Canine Mammary Gland: a Histological Type or Tumour Cell Arrangement? J Comp Pathol. 2022 Jan;190: 1–12.
6.Sleeckx N, de Rooster H, Veldhuis Kroeze E, Van Ginneken C, Van Brantegem L. Canine Mammary Tumours, an Overview. Reproduction in Domestic Animals. 2011 Dec 6;46(6):1112–31.
7.Nosalova N, Huniadi M, Horňáková I', Valenčáková A, Horňák S, Nagoos K, et al. Canine Mammary Tumors: Classification, Biomarkers, Traditional and Personalized Therapies. Int J Mol Sci. 2024 Mar 1;25(5):2891.
8.Tamarindo GH, Novais AA, Chuffa LGA, Zuccari DAPC. Metabolic Alterations in Canine Mammary Tumors. Animals. 2023 Aug 30;13(17):2757.
9.Vinothini G, Balachandran C, Nagini S. Evaluation of Molecular Markers in Canine Mammary Tumors: Correlation With Histological Grading. Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics. 2009 May 1;18(5):193–201.
10.Kim TM, Yang IS, Seung BJ, Lee S, Kim D, Ha YJ, et al. Cross-species oncogenic signatures of breast cancer in canine mammary tumors. Nat Commun. 2020 Jul 17;11(1):3616.
11.Brandão Y de O, Toledo MB, Chequin A, Cristo TG, Sousa RS, Ramos EAS, et al. DNA Methylation Status of the Estrogen Receptor α Gene in Canine Mammary Tumors. Vet Pathol. 2018 Jul 22;55(4):510–6.
12.Biondi LR, Tedardi MV, Gentile LB, Chamas PPC, Dagli MLZ. Quantification of Global DNA Methylation in Canine Mammary Gland Tumors via Immunostaining of 5-Methylcytosine: Histopathological and Clinical Correlations. Front Vet Sci. 2021 Feb 25;8.
13.Marei HE. Epigenetic regulators in cancer therapy and progression. NPJ Precis Oncol. 2025 Jun 28;9(1):206.
14.Qureshi SA, Bashir MU, Yaqinuddin A. Utility of DNA methylation markers for diagnosing cancer. Int J Surg. 2010;8(3):194–8.
15.Biondi LR, Tedardi MV, Gentile LB, Chamas PPC, Dagli MLZ. Quantification of Global DNA Methylation in Canine Mammary Gland Tumors via Immunostaining of 5-Methylcytosine: Histopathological and Clinical Correlations. Front Vet Sci. 2021;8:628241.
16.Quan S, Huang H. Epigenetic contribution to cancer. In 2024. p. 1–25.
17.Van Tongelen A, Loriot A, De Smet C. Oncogenic roles of DNA hypomethylation through the activation of cancer-germline genes. Cancer Lett. 2017 Jun;396:130–7.
18.Baylin SB, Jones PA. Epigenetic Determinants of Cancer. Cold Spring Harb Perspect Biol. 2016 Sep;8(9).
19.Atalay C. Epigenetics in breast cancer. Exp Oncol. 2013 Dec;35(4):246–9.
20.Liehr T. Repetitive Elements in Humans. Int J Mol Sci. 2021 Feb;22(4).
21.Böhne A, Brunet F, Galiana-Arnoux D, Schultheis C, Volff JN. Transposable elements as drivers of genomic and biological diversity in vertebrates. Chromosome Res. 2008;16(1):203–15.
22.Kitkumthorn N, Mutirangura A. Long interspersed nuclear element-1 hypomethylation in cancer: biology and clinical applications. Clin Epigenetics. 2011 Aug;2(2):315–30.
23.Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998 Jul;15(7):539–53.
24.Pfeifer GP. Defining Driver DNA Methylation Changes in Human Cancer. Int J Mol Sci. 2018 Apr;19(4).
25.Goldschmidt M, Peña L, Rasotto R, Zappulli V. Classification and Grading of Canine Mammary Tumors. Vet Pathol. 2011 Jan 25;48(1):117–31.
26.de Faria Lainetti P, Brandi A, Leis Filho AF, Prado MCM, Kobayashi PE, Laufer-Amorim R, et al. Establishment and Characterization of Canine Mammary Gland Carcinoma Cell Lines With Vasculogenic Mimicry Ability in vitro and in vivo. Front Vet Sci. 2020;7:583874.
27.González-Andrade F, Sánchez D. DNA typing from skeletal remains following an explosion in a military fort--first experience in Ecuador (South-America). Leg Med (Tokyo). 2005 Oct;7(5):314–8.
28.Nestor CE, Ottaviano R, Reddington J, Sproul D, Reinhardt D, Dunican D, et al. Tissue type is a major modifier of the

5-hydroxymethylcytosine content of human genes. Genome Res. 2012 Mar;22(3):467–77.
29.Bhutta ZA, Choi KC. Canine mammary tumors as a promising adjunct preclinical model for human breast cancer research: similarities, opportunities, and challenges. Arch Pharm Res. 2025 Jan 3;48(1):43–61.
30.Gray M, Meehan J, Martínez-Pérez C, Kay C, Turnbull AK, Morrison LR, et al. Naturally-Occurring Canine Mammary Tumors as a Translational Model for Human Breast Cancer. Front Oncol. 2020 Apr 28;10.
31.Lee KH, Shin TJ, Kim WH, Cho JY. Methylation of LINE-1 in cell-free DNA serves as a liquid biopsy biomarker for human breast cancers and dog mammary tumors. Sci Rep. 2019 Jan 17;9(1):175.
32.Dias ML de M, Andrade JML, de Castro MB, Galera PD. Survival analysis of female dogs with mammary tumors after mastectomy: Epidemiological, clinical and morphological aspects. Pesquisa Veterinaria Brasileira. 2016;36(3):181–6.
33.Saito K, Kawakami K, Matsumoto I, Oda M, Watanabe G, Minamoto T. Long interspersed nuclear element 1 hypomethylation is a marker of poor prognosis in stage IA non-small cell lung cancer. Clin Cancer Res. 2010 Apr;16(8):2418–26.
34.Kemp JR, Longworth MS. Crossing the LINE Toward Genomic Instability: LINE-1 Retrotransposition in Cancer. Front Chem. 2015;3:68.
35.Chalitchagorn K, Shuangshoti S, Hourpai N, Kongruttanachok N, Tangkijvanich P, Thong-ngam D, et al. Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis. Oncogene. 2004 Nov;23(54):8841–6.
36.Cruickshanks HA, Tufarelli C. Isolation of cancer-specific chimeric transcripts induced by hypomethylation of the LINE-1 antisense promoter. Genomics. 2009 Dec;94(6):397–406.
37.Lee KH, Shin TJ, Kim WH, Cho JY. Methylation of LINE-1 in cell-free DNA serves as a liquid biopsy biomarker for human breast cancers and dog mammary tumors. Sci Rep. 2019 Jan;9(1):175.
38.Anwar SL, Hasemeier B, Schipper E, Vogel A, Kreipe H, Lehmann U. LINE-1 hypomethylation in human hepatocellular carcinomas correlates with shorter overall survival and CIMP phenotype. PLoS One. 2019;14(5):e0216374.
39.Barchitta M, Quattrocchi A, Maugeri A, Vinciguerra M, Agodi A. LINE-1 hypomethylation in blood and tissue samples as an epigenetic marker for cancer risk: a systematic review and meta-analysis. PLoS One. 2014;9(10):e109478.
40.Swets M, Zaalberg A, Boot A, van Wezel T, Frouws MA, Bastiaannet E, et al. Tumor LINE-1 Methylation Level in Association with Survival of Patients with Stage II Colon Cancer. Int J Mol Sci. 2016 Dec;18(1).
41.Ardeljan D, Taylor MS, Ting DT, Burns KH. The Human Long Interspersed Element-1 Retrotransposon: An Emerging Biomarker of Neoplasia. Clin Chem. 2017 Apr;63(4):816–22.
42.Harada K, Baba Y, Ishimoto T, Chikamoto A, Kosumi K, Hayashi H, et al. LINE-1 methylation level and patient prognosis in a database of 208 hepatocellular carcinomas. Ann Surg Oncol. 2015 Apr;22(4):1280–7.
43.Min J, Choi B, Han TS, Lee HJ, Kong SH, Suh YS, et al. Methylation Levels of LINE-1 As a Useful Marker for Venous Invasion in Both FFPE and Frozen Tumor Tissues of Gastric Cancer. Mol Cells. 2017 May;40(5):346–54.
44.Stricker I, Tzivras D, Nambiar S, Wulf J, Liffers ST, Vogt M, et al. Site- and grade-specific diversity of LINE1 methylation pattern in gastroenteropancreatic neuroendocrine tumours. Anticancer Res. 2012 Sep;32(9):3699–706.

Observação: os/(as) autores/(as) declaram não existir conflitos de interesses de qualquer natureza.