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Epigenetic alterations in canine mammary cancer

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Abstract

In dogs, mammary cancer is the most common tumor type, especially in unspayed females. 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. Epigenetic mechanisms are responsible for gene expression modulation without alterations in the DNA sequence and include but are not limited to DNA methylation, histone modifications, and noncoding RNAs. Epigenetic patterns are known to influence a variety of biological mechanisms, such as cellular differentiation and development, and dysregulations of those patterns may result in several diseases, such as cancer. In this respect, this review summarizes the main findings concerning epigenetic alterations in canine mammary cancer, their relationship with the carcinogenic process, and their use as diagnostic and prognostic markers.

Keywords: Histone modifications, DNA methylation, ncRNA, biomarkers, comparative oncology.

Introduction

As in humans, mammary cancer is the most frequent cancer diagnosed among female dogs, especially unspayed dogs (Markkanen, 2019; Mohammed *et al.*, 2020). This could be partly explained by dogs being a companion animal, sharing similar environmental conditions with humans (Rybicka *et al.*, 2015; Bulkowska *et al.*, 2017; Jeong *et al.*, 2019). In addition, both species share several mammary cancer risk factors and biological patterns, including aging, hormonal exposure, obesity in early life, treatment response (due to similar P450 cytochrome activity), and spontaneous development (Rybicka *et al.*, 2015; Bulkowska *et al.*, 2017; Markkanen, 2019; Xavier *et al.*, 2019; Beetch *et al.*, 2020).

Epigenetic mechanisms result in changes in gene expression without alterations in the DNA sequence (Inbar-Feigenberg *et al.*, 2013; Zhou *et al.*, 2021), influencing a variety of biological phenomena, such as cellular differentiation and development, metabolism, phenotypic variability, inheritance, evolution, behavior, and several diseases, such as cancer (Nicoglou and Merlin, 2017; Zhou *et al.*, 2021). The regulatory mechanisms of epigenetic modifications include DNA methylation, RNA interference, histone modifications (Figure 1) (Zhou *et al.*, 2021), DNA–protein interactions, chromatin accessibility and tridimensional structure (Casado-Pelaez *et al.*, 2022).

Although mutations in many genes, such as in *BRCA1*, *BRCA2*, and *TP53* (Markkanen, 2019; Beetch *et al.*, 2020), as well as changes in several mammary cancer-related pathways, such as *KRAS*, *PTEN*, and *MAPK* (Bulkowska *et al.*, 2017), have already been described for both species, the genetic

influence in the genesis and development of this tumor and information about epigenetic alterations in canine mammary cancer are still scarcely known. The aim of this review is to summarize the main findings concerning epigenetic alterations in canine mammary cancer, their relationships with carcinogenesis and their use as diagnostic and prognostic markers.

DNA methylation

DNA methylation is a chemical modification characterized by the covalent transfer of a methyl group at the C5 position of a cytosine base, producing 5-methylcytosine (Skvortsova *et al.*, 2019). This modification occurs mainly at clusters of cytosine–guanine dinucleotides (CpG), named CpG islands (CGIs); is catalyzed by an enzymatic family called DNA methyltransferases (DNMTs); and plays an important role in gene expression regulation, genomic imprinting, X chromosome inactivation, retroelement silencing, and genome stability (Nishiyama and Nakanishi, 2021).

In normal cells, methylation is necessary for maintaining cell growth and metabolism, whereas an abnormal DNA methylation pattern can lead to diseases, such as cancers (Zhou *et al.*, 2021). In cancer, hypermethylation of CGIs is common and frequently observed in transcriptional regulatory regions, such as promoters of tumor suppressor genes (TSGs), and is associated with the silencing of genes controlling cell growth and related pathways (Figure 2) (Skvortsova *et al.*, 2019). Nevertheless, a DNA hypermethylation pattern is observed in a tissue-specific manner (Nishiyama and Nakanishi, 2021).

Although CpG dinucleotides are largely underrepresented in mammalian genomes, dogs have the largest number of CGIs and the highest CGI density among several mammalian genomes studied, such as humans, cows, and rats (Han and Zhao, 2009). Approximately 30,000 CGIs have been identified in the human genome (Jeziorska *et al.*, 2017), whereas 58,327 CGIs have been mapped in the dog genome (Han and Zhao, 2009),

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with 2.2% of them annotated in the promoter and exon regions (Jeong *et al.*, 2019), thus suggesting that DNA methylation must have some relevance on gene expression regulation in the dog genome.

To determine the influence of methylation in canine mammary carcinogenesis, Nam *et al.* (2020) analyzed only the CMT-associated genome-wide methylation signature using methyl CpG binding domain sequencing, observing that differentially methylated regions (DMRs) were distributed similarly on CGIs and tended to be enriched in gene regions. The authors described a hypermethylated pattern in tumoral tissues compared with their normal counterparts. In addition, hypermethylated regions were observed in TSGs, whereas oncogenes are frequently hypomethylated, especially in intronic regions, a result that is correlated with gene expression and with human breast cancer (Nam *et al.*, 2020).

A similar approach using the identification of DMRs to map genes subject to methylation modulation was applied by Schabort *et al.* (2020), which resulted in a mapping of 16,061 differentially methylated genes. Among them, two genes, *ANK2* and *EPAS1*, were observed to have a high level of hypermethylation in tumoral tissues compared with normal samples. In addition, a reverse correlation between the hypermethylation status and gene expression was described, suggesting that the expression levels of those genes are affected by their methylation status. Although both genes could be used as biomarkers in mammary tissues, in plasma samples this same profile was observed only for *ANK2*, similar to the described for human breast cancer, highlighting its potential as a diagnostic marker in liquid biopsy.

Although the classification in molecular subtypes is not frequently observed in CMTs, in a work focusing on the triple-negative (TN) subtype of mammary cancer, Beetch *et al.* (2020) analyzed samples of different tumor stages as well as healthy donors, described the hypermethylation pattern of cancer-related genes, and found that those involved in transcriptional regulation, apoptosis, signal transduction, and cell migration are associated with gene expression and progression of the disease in dogs, suggesting that the methylation pattern has the potential to be utilized in clinics to distinguish progression stages in TN cancers.
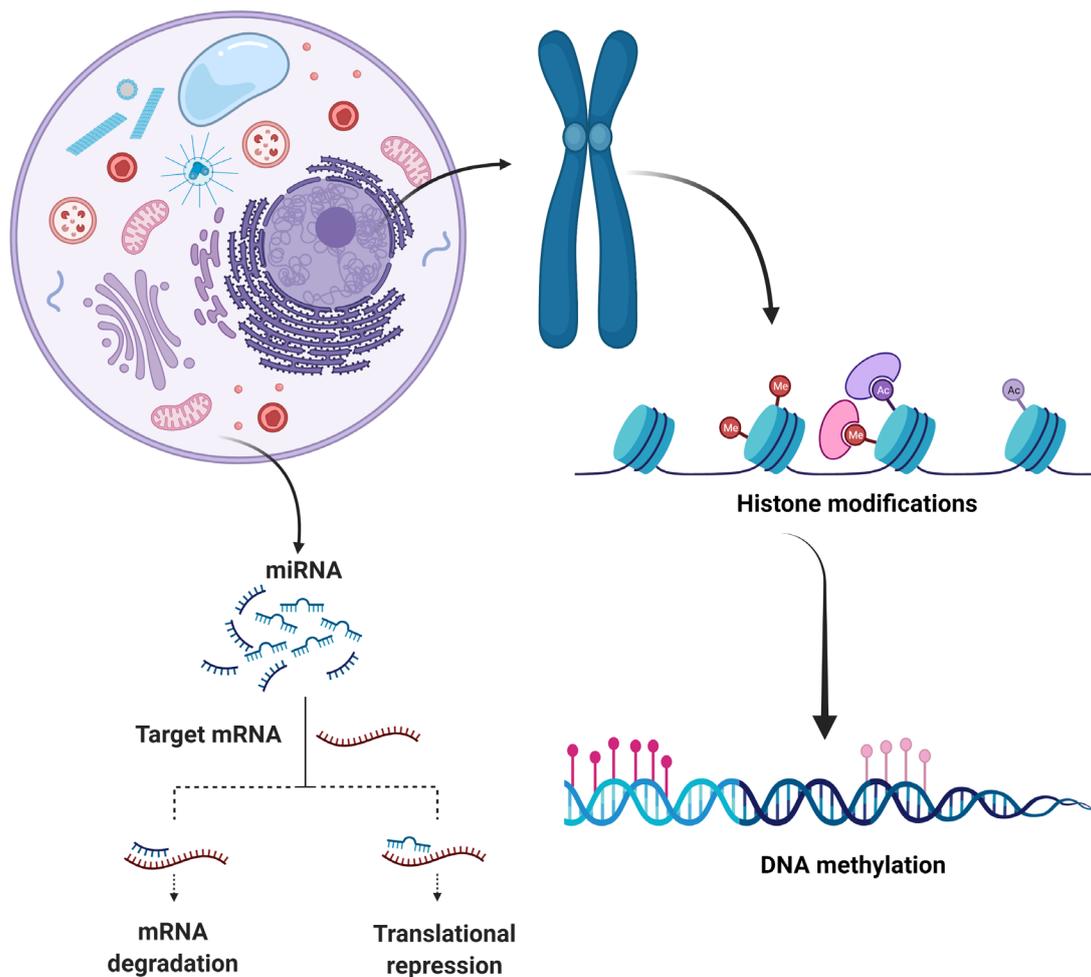
Figure 1 – Main epigenetic mechanisms studied in canine mammary cancer: histone modifications, DNA methylation and miRNA expression. miRNAs may affect gene expression in two ways depending on their complementarity with the target messenger RNA (mRNA): perfect complementation results in mRNA cleavage, while partial complementation leads to translational repression.

In a broader analysis, Biondi *et al.* (2021), who analyzed the global genome methylation pattern of canine mammary tumors by immunohistochemistry, described a lower hypermethylation pattern for malignant samples, which is associated with tumor recurrence. In the same way, in mammary tissues as well as in plasma, Lee *et al.* (2019) observed a low methylation level in LINE-1, a retrotransposon, in benign and tumor samples when compared with healthy samples. Although these results might seem contradictory with the previous ones, they are in accordance with the global hypomethylated and regional hypermethylated patterns in tumors during their initiation and progression.

Meanwhile, studies on mammary cancer evaluating the methylation pattern of single genes did not find any evidence of methylation affecting gene expression regulation, as described for *BRCA1* (Qiu and Lind, 2016; Ferreira *et al.*, 2019), *ESR1* (Brandão *et al.*, 2018) and *P15/CDKN2B* (Faro *et al.*, 2018). These results differ slightly from those observed in human counterparts. For *CDKN2B*, there are still contradictory results, ranging from no correlation between promoter hypermethylation and breast cancer risk (Qi and Xiong, 2018) to an early and frequent event of breast carcinogenesis (Jung *et al.*, 2013). The hypermethylation of the promoter regions of *BRCA1* (Jung *et al.*, 2013; Harahap *et al.*, 2018; Vu *et al.*, 2018) and *ESR1* (Sun *et al.*, 2017; Kirn *et al.*, 2018), which are considered biomarkers, is associated with breast carcinogenesis. On the other hand, Ren *et al.* (2018), who analyzed the methylation pattern of *DAPK1* and *MGMT* genes in clinical samples, observed an increase in methylation status according to tumor aggressiveness, suggesting their use as

prognostic and diagnostic markers, as observed in humans (An *et al.*, 2017; Yadav *et al.*, 2018; Ghalkhani *et al.*, 2021; Yari *et al.*, 2022).

The alterations observed in cancer methylation patterns may be caused by DNMT expression dysregulation; thus, several anticancer drugs targeting DNMTs, such as the DNA methyltransferase inhibitor 5-azacitydine (5-AzaC), are approved for clinical use to treat tumors, such as acute myelodysplastic leukemia (Hillyar *et al.*, 2020). In humans, 5-AzaC has been shown to inhibit the invasiveness and growth of several human breast cancer cell lines, and its potential as a therapeutic agent in canine mammary cancer was demonstrated by Harman *et al.* (2016), suggesting that more studies concerning the use of 5-AzaC in canine mammary cancer should be developed.

Histone modifications

One of the features observed in eukaryotic cells is that, in the nucleus, DNA is packaged in the form of chromatin, a macromolecular complex of DNA and histone proteins (Dawson and Kouzarides, 2012). The chromatin has a basic unit called a nucleosome, which is formed by 147 base pairs of DNA wrapped around a histone octamer (comprising two of each histone: H2A, H2B, H3, and H4). The protruding N-terminal amino acid tails of core histones (especially H3 and H4) are subject to posttranslational modifications (PTMs), including methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and ADP ribosylation, which can regulate the chromatin structure and remodel it, resulting in two structural and transcriptional states according to the
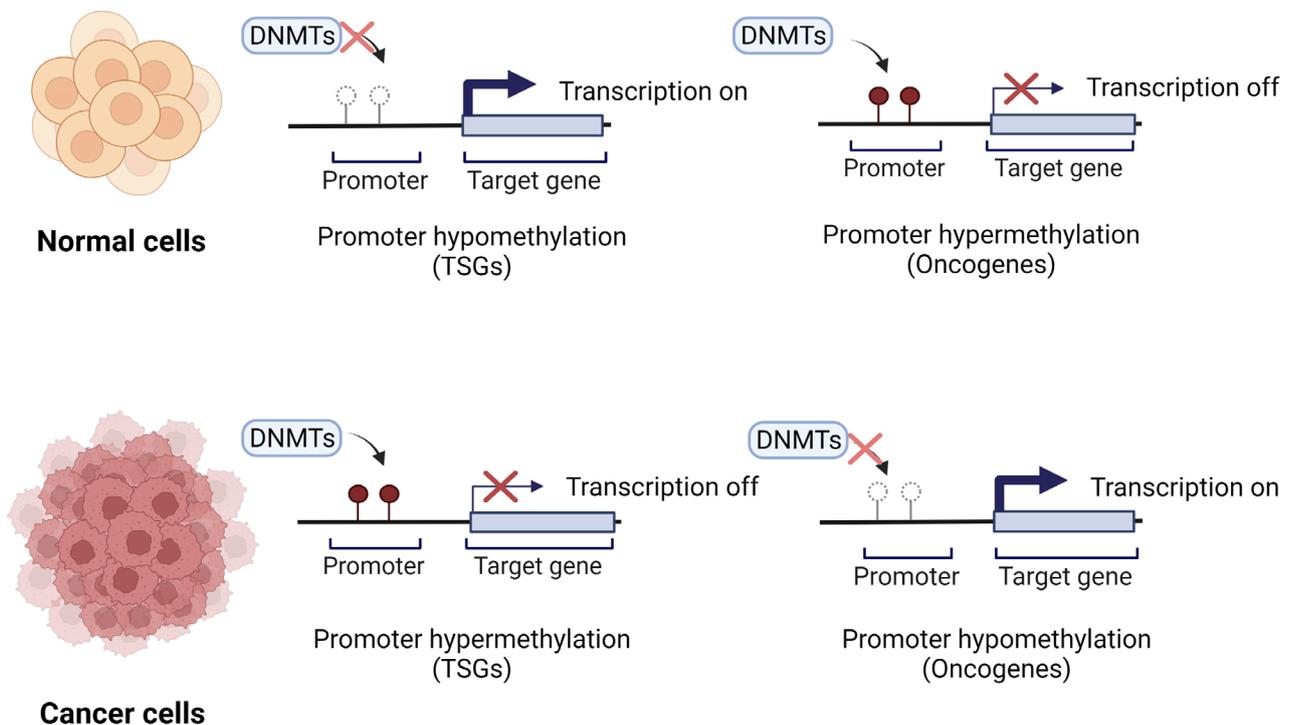

Figure 2 – DNA methylation pattern in different cell types. In normal cells, the promoter regions of tumor suppressor genes (TSGs), as cell cycle regulators, are hypomethylated; meanwhile, in oncogenes (as transcription factors), this same region is frequently hypermethylated by DNA methyltransferases (DNMTs). On the other hand, an opposite pattern is described in cancer cells, with TSGs being hypermethylated, while oncogenes present a hypomethylated promoter region. Adapted from "Epigenetic deregulation in cancer", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

noncovalent interactions within and between nucleosomes: an active and a repressive state (Dawson and Kouzarides, 2012; Zhang *et al.*, 2015; Ilango *et al.*, 2020; Boyson *et al.*, 2021).

While the repressive states are formed by supercoiled structures enriched for DNA and histone methylation marks (such as H3 trimethylation of lysine 27 and lysine 9), resulting in closed chromatin (heterochromatin), active states are accessible to transcription factors and are enriched for histone marks, such as high levels of lysine acetylation on the H3 and H4 tails, which forms open chromatin (euchromatin) frequently associated with an active transcriptional pattern (Figure 3) (Biswas and Rao, 2018; Nebbioso *et al.*, 2018). These patterns of histone marks are established through a dynamic interplay between protein machineries that recognize, add, and remove these PTMs, named histone readers, writers, and erasers, respectively (Gillette and Hill, 2015; Zhang *et al.*, 2015; Uckelmann and Davidovich, 2021).

Histone writers, such as histone methyltransferases (HMTs) and histone acetyltransferases (HATs), are a group of enzymes capable of transferring chemical compounds, such as methyl and acetyl groups, to the N-terminal tail of histones. These covalent modifications are thus recognized and interpreted by histone readers, such as BET proteins, which are capable of identifying and binding due to the presence of specialized domains, inducing chromatin structural changes, recruiting other chromatin modifiers, or providing scaffold proteins for different nuclear processes, such as transcription, replication, or repair. On the other hand, those PTMs may be removed by enzymes known as erasers, such as histone demethylases (HDMs) and histone deacetylases (HDACs) (Biswas and Rao, 2018; López *et al.*, 2022).

These modifications play an important role in human breast cancer, providing specific chromatin signatures for each molecular subtype (Xi *et al.*, 2018), and could be considered prognostic (Elsheikh *et al.*, 2009; Fath *et al.*, 2022) and therapeutic (Sharda *et al.*, 2020; Wang *et al.*, 2020) markers. In canine mammary cancer, as stated by Liu *et al.* (2014), 35 downregulated chromatin-modifier genes, mostly related to histone active and repressive modifiers (i.e., associated with methylation, demethylation, acetylation, deacetylation, ubiquitination, and deubiquitination modifications), have been described in complex carcinomas with myoepithelial cell proliferation, suggesting that this tumor type originates mainly from epigenomic rather than genomic alterations.

BET proteins are a family of epigenetic readers characterized by two tandem N-terminal BRD regions followed by an extraterminal domain, which bind acetylated histones, modulate chromatin architecture and recruit transcriptional regulators (Sahni and Keri, 2018; Hill *et al.*, 2021; Neganova *et al.*, 2022). The use of BET protein inhibitors, already described as potential therapeutic agents in human breast cancer (Andrikopoulou *et al.*, 2020; Khandekar and Tiriveedhi, 2020), is also a promising therapy in canine mammary cancer, as they can downregulate several genes related to self-renewal pathways, such as WNT, NOTCH, Hedgehog, and PI3K/AKT/mTOR (Xavier *et al.*, 2019).

Meanwhile, analysis of histone modifications considering different tumor grades obtained contrasting results. Although Liu *et al.* (2014) did not correlate their findings with tumor grade, Vinothini *et al.* (2009) described significantly increased expression in histone deacetylase 1, a chromatin-modifier eraser protein, in tumors in more advanced states (grade III)
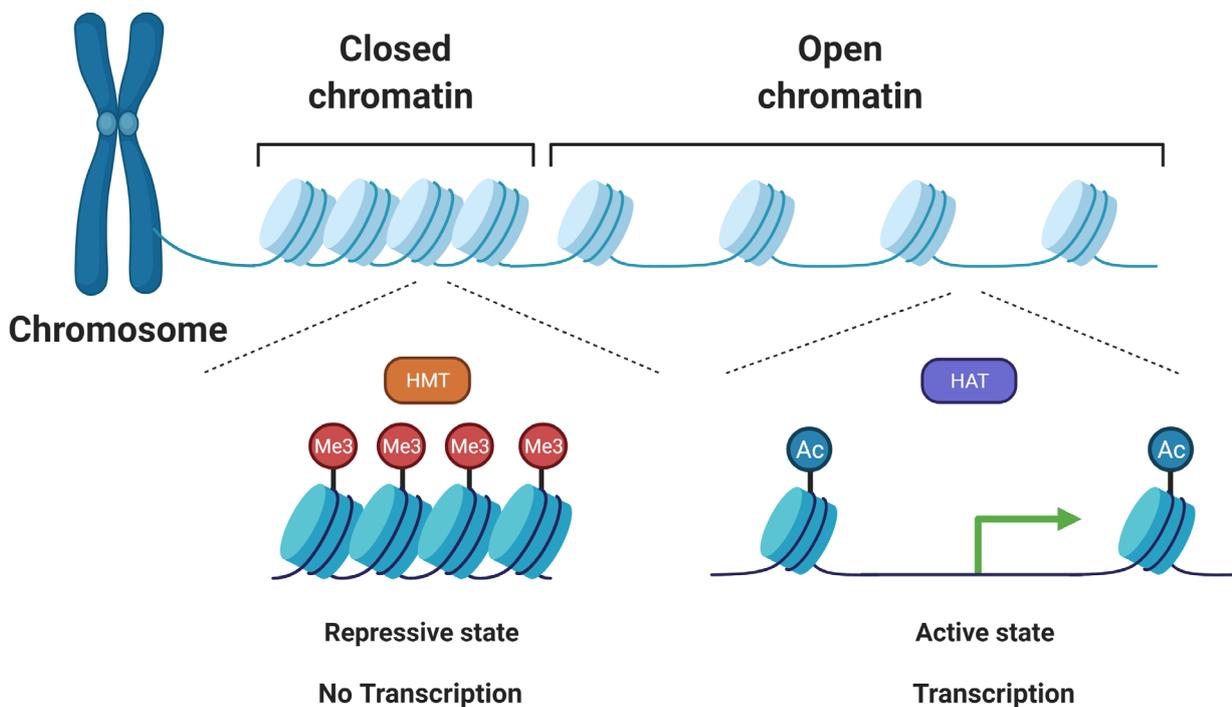

Figure 3 – Histone modifications and their impact on chromatin remodeling. Histone methylation is catalyzed by histone methyltransferases (HMTs), resulting in chromatin packaging (heterochromatin) and a repressive state (no transcription). Conversely, histone acetylation by histone acetyltransferases (HATs) results in open chromatin (euchromatin) associated with an active transcriptional pattern. Adapted from "Epigenetics and gene expression", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

than in those in more initial states (grades I and II). Similar results were described for EZH2, the catalytic subunit of the epigenetic regulator Polycomb repressive complex 2, a protein complex involved in gene silencing through the trimethylation of histone 3 lysine residue 27, where EZH2 expression increases with the malignancy grade (Choi *et al.*, 2016), suggesting that this alteration may also influence the prognosis of the patient. Although not related to tumor grade, EZH2 upregulation in human breast cancer is associated with a poor prognosis and is a potential target for therapeutic options (Xie *et al.*, 2022; Zhang *et al.*, 2022).

Noncoding RNAs

Noncoding RNAs (ncRNAs) are regulatory players of gene expression classified according to their size and function, which includes small interfering RNAs, microRNAs (miRNAs), and long ncRNAs (lncRNAs), playing important roles in gene expression regulation at several levels: transcription, mRNA degradation, splicing, and translation (Inbar-Feigenberg *et al.*, 2013).

miRNAs are short single-stranded ncRNAs approximately 17–24 nucleotides in length that posttranscriptionally regulate gene expression and are involved in the regulation of several cellular pathways, such as differentiation, proliferation, and apoptosis (Heishima *et al.*, 2017; Loh *et al.*, 2019; Pasquini and Kunej, 2019). Dysregulation of a single miRNA or a small subset of miRNAs can therefore have a significant impact on cellular outcomes and sometimes induce the development of several disease processes, such as cancer (Loh *et al.*, 2019). Considering their dysregulation during tumorigenesis,

miRNAs can be subdivided into two main classes: oncogenic miRNAs (oncomiRs), usually upregulated and responsible for suppressing the expression of TSGs, and tumor suppressor miRNAs, a class that inhibits the expression of oncogenes and is usually downregulated in cancer (Loh *et al.*, 2019). However, some miRNAs may play a dual role depending on the tumor site (Kolenda *et al.*, 2020).

One feature of cancer cells is that they can release their miRNAs into the bloodstream. Such "circulating miRNAs" are stabilized by either their binding proteins or extracellular vesicles, such as exosomes and microvesicles, which makes them resistant to RNase degradation. As the levels of circulating miRNAs accurately reflect the number of tumor cells, response to treatment, clinical stages, and tumor grades, circulating miRNAs have a great potential to be used as diagnostic and prognostic biomarkers in neoplastic diseases (Heishima *et al.*, 2017).

Due to its relevance in cellular pathways, miRNA expression is the most studied epigenetic alteration in canine mammary cancer and is considered a promising biomarker (Table 1). However, in this review, we will focus on the most studied miRNAs and their relevance to canine mammary tumorigenesis.

One of the most studied miRNAs is miR-21, which is described as an oncomiR in humans and is involved in cell migration, invasion, metastasis, and apoptosis (Nurzadeh *et al.*, 2021). Although its characterization in a cell line derived from a grade II mammary tumor suggests a low expression of miR-21 (Raposo *et al.*, 2017), the same pathogenic role described for humans was observed by Boggs *et al.* (2008)

Table 1 – miRNA expression pattern in clinical samples of canine mammary cancer and their possible application on veterinary routine. CL= cell line; P= plasma; S= serum; T= mammary tissue.

miRNA	Sample type	Expression	Clinical application	References
miR-18a	S	Up-regulated	Prognostic	Fish *et al.* (2020)
miR-19b	S	Up-regulated	Diagnostic	Fish *et al.* (2020)
miR-21	T/S	Up-regulated	Diagnostic and prognostic	Boggs *et al.* (2008); von Deetzen *et al.* (2014); Bulkowska *et al.* (2017); Jain *et al.* (2021); Ramadan *et al.* (2021)
miR-29b	T/S	Down-regulated	Diagnostic and prognostic	Bulkowska *et al.* (2017); Jain *et al.* (2021)
	T/S	Up-regulated	Diagnostic	Boggs *et al.* (2008); Fish *et al.* (2020)
miR-96	T/S	Up-regulated	Diagnostic	Jeong *et al.* (2019)
miR-101	T/S	Down-regulated	Diagnostic	von Deetzen *et al.* (2014); Bulkowska *et al.* (2017)
miR-125a	T/S	Down-regulated	Prognostic	von Deetzen *et al.* (2014); Bulkowska *et al.* (2017)
	S	Up-regulated	Diagnostic	Fish *et al.* (2020)
miR-126	P	Up-regulated	Diagnostic	Heishima *et al.* (2017)
miR-143	T/S	Down-regulated	Prognostic	von Deetzen *et al.* (2014); Bulkowska *et al.* (2017)
miR-145	T/S	Down-regulated	Prognostic	von Deetzen *et al.* (2014); Bulkowska *et al.* (2017)
miR-149	T/S	Down-regulated	Diagnostic	Jeong *et al.* (2019)
miR-194	T	Up-regulated	Diagnostic	von Deetzen *et al.* (2014)
miR-195	T/CL	Down-regulated	Diagnostic	Zhang *et al.* (2021)
miR-210	T/S	Down-regulated	Diagnostic	von Deetzen *et al.* (2014); Bulkowska *et al.* (2017)
miR-214	P	Up-regulated	Diagnostic	Heishima *et al.* (2017)
miR-497	T/CL	Down-regulated	Diagnostic	Zhang *et al.* (2021)
miR-8832	T/S	Up-regulated	Diagnostic	Jeong *et al.* (2019)

and von Deetzen *et al.* (2014), who reinforced the oncogenic role of this miRNA in mammary cancer. Therefore, different studies have analyzed miR-21 expression in the serum samples of patients with mammary cancer, comparing the results with either or both patients who are healthy and with benign tumors. Overall, overexpression of miR-21 is observed in patients with cancer, supporting its use as a diagnostic and prognostic marker using minimally invasive methods (Hannafon *et al.*, 2016; Jain *et al.*, 2021; Ramadan *et al.*, 2021).

Another canine mammary cancer studied is miR-18a, which is upregulated in human breast cancer, although it can be downregulated in other tumor types, such as gastric cancer (Kolenda *et al.*, 2020). In canines, this miRNA is an oncomiR that is upregulated in exosomes isolated from mammary cell lines (Fish *et al.*, 2018) and in body fluids of tumoral patients, where it is also associated with lymph node invasion (Fish *et al.*, 2020), suggesting its use as a diagnostic and prognostic marker in liquid biopsy. This miRNA may be involved in other levels of epigenetic regulation, as several of their gene targets are related to chromatin remodeling processes, such as methylation, acetylation, and ubiquitination of histones (Fish *et al.*, 2018).

Although the majority of studies are in agreement with the function of the miRNAs in canine mammary cancer, controversial results exist in some cases; for example, miR-29b is downregulated in the FR37-CMT cell line (Raposo *et al.*, 2017) and in tumoral tissues of canine patients (Jain *et al.*, 2021) but upregulated in the SNP cell line (Osaki *et al.*, 2016), tumoral tissues (Boggs *et al.*, 2008), and serum (Fish *et al.*, 2020) of patients.

lncRNAs are molecules with at least 200 nucleotides transcribed mainly by RNA polymerase II that do not encode proteins and can influence gene expression at the transcriptional and posttranscriptional levels, influencing several cellular processes, such as proliferation, differentiation and apoptosis (Naz *et al.*, 2021; Smolarz *et al.*, 2021). Despite a few studies in the area, lncRNAs have great potential as predictive markers of canine mammary cancer. Xu *et al.* (2021) reported that the overexpression of lnc-42060 in tumoral tissues and mammary cancer cell lines is associated with cell proliferation, migration, and increased tamoxifen resistance. A similar result was described by Lu *et al.* (2022), who analyzed cell lines and tissues of patients with mammary cancer and described the influence of two lncRNAs (the downregulated expression of lnc-40589 and overexpressed lnc-34977) on cell proliferation, migration, and invasion in mammary cancer, suggesting a key role during carcinogenesis.

Conclusion

Although canine studies are still scarce compared with those of human counterparts, the studies approaching the epigenetic aspects of canine mammary cancer reinforced the importance of canine mammary carcinogenesis. Several described epigenetic alterations, especially those involving ncRNAs, have potential as predictive, diagnostic, and prognostic markers for canine mammary cancer, including its use in liquid biopsy. These biomarkers may be further used in clinical routines and likely help veterinary doctors proceed on a faster and more accurate diagnosis and choose the best treatment option for their patients. Considering the

above, larger and more detailed studies in the epigenetic field are urgently needed to better understand the influence of this area on canine mammary tumors.

Conflict of Interest

No potential conflict of interest was reported by the author.

Author Contributions

BNB conceived and wrote the manuscript. The author read and approved the final version.

References

An N, Shi Y, Ye P, Pan Z and Long X (2017) Association between MGMT promoter methylation and breast cancer: A meta-analysis. Cell Physiol Biochem 42:2430-2440.

Andrikopoulou A, Liontos M, Koutsoukos K, Dimopoulos M-A and Zagouri F (2020) The emerging role of BET inhibitors in breast cancer. Breast 53:152-163.

Beetch M, Harandi-Zadeh S, Yang T, Boycott C, Chen Y, Stefanska B and Mohammed S (2020) DNA methylation landscape of triple-negative ductal carcinoma in situ (DCIS) progressing to the invasive stage in canine breast cancer. Sci Rep 10:2415.

Biondi LR, Tedardi MV, Gentile LB, Chamas PPC and Dagli MLZ (2021) Quantification of global DNA methylation in canine mammary gland tumors via immunostaining of 5-methylcytosine: Histopathological and clinical correlations. Front Vet Sci 8:628241.

Biswas S and Rao CM (2018) Epigenetic tools (The Writers, The Readers and The Erasers) and their implications in cancer therapy. Eur J Pharmacol 837:8-24.

Boggs RM, Wright ZM, Stickney MJ, Porter WW and Murphy KE (2008) MicroRNA expression in canine mammary cancer. Mamm Genome 19:561-569.

Boyson SP, Gao C, Quinn K, Boyd J, Paculova H, Frietze S and Glass KC (2021) Functional roles of bromodomain proteins in cancer. Cancers (Basel) 13:3606.

Brandão YO, Toledo MB, Chequin A, Cristo TG, Sousa RS, Ramos EAS and Klassen G (2018) DNA methylation status of the estrogen receptor α gene in canine mammary tumors. Vet Pathol 55:510-516.

Bulkowska M, Rybicka A, Senses KM, Ulewicz K, Witt K, Szymanska J, Taciak B, Klopfleisch R, Hellmén E, Dolka I *et al.* (2017) MicroRNA expression patterns in canine mammary cancer show significant differences between metastatic and non-metastatic tumours. BMC Cancer 17:728.

Casado-Pelaez M, Bueno-Costa A and Esteller M (2022) Single cell cancer epigenetics. Trends Cancer 8:820-838.

Choi H-J, Jang S, Ryu J-E, Lee H-J, Lee H-B, Ahn W-S, Kim H-J, Lee H-J, Lee HJ, Gong G-Y *et al.* (2016) Significance of EZH2 expression in canine mammary tumors. BMC Vet Res 12:164.

Dawson MA and Kouzarides T (2012) Cancer epigenetics: From mechanism to therapy. Cell 150:12-27.

Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM, Soria D, Garibaldi JM, Paish CE, Ammar AA *et al.* (2009) Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. Cancer Res 69:3802-3809.

Faro TAS, Pinheiro RDDR, Calcagno DQ, Pereira WLA, De Aguirra LRVM, Burbano RR, Harada ML and Borges BDN (2018) Expression pattern of Cdkn2b and its regulators in canine mammary tumors. Anticancer Res 38:6333-6338.

Fath MK, Azargoonjahromi A, Kiani A, Jalalifar F, Osati P, Oryani MA, Shakeri F, Nasirzadeh F, Khalesi B, Nabi-Afjadi M *et al.* (2022) The role of epigenetic modifications in drug resistance and treatment of breast cancer. Cell Mol Biol Lett 27:52.

Ferreira VC, Pinheiro DR, Sousa RM, Aguirra LRVM, Pereira WLA, Burbano RMR and Borges BN (2019) Methylation pattern and mutational status of BRCA1 in canine mammary tumors in a Brazilian population. Comp Clin Path 28:63-67.

Fish EJ, Irizarry KJ, DeInnocentes P, Ellis CJ, Prasad N, Moss AG and Bird RC (2018) Malignant canine mammary epithelial cells shed exosomes containing differentially expressed microRNA that regulate oncogenic networks. BMC Cancer 18:832.

Fish EJ, Martinez-Romero EG, DeInnocentes P, Koehler JW, Prasad N, Smith AN and Bird RC (2020) Circulating microRNA as biomarkers of canine mammary carcinoma in dogs. J Vet Intern Med 34:1282-1290.

Ghalkhani E, Akbari MT, Izadi P, Mahmoodzadeh H and Kamali F (2021) Assessment of DAPK1 and CAVIN3 gene promoter methylation in breast invasive ductal carcinoma and metastasis. Cell J 23:397-405.

Gillette TG and Hill JA (2015) Readers, writers, and erasers: Chromatin as the whiteboard of heart disease. Circ Res 116:1245-1253.

Han L and Zhao Z (2009) Contrast features of CpG islands in the promoter and other regions in the dog genome. Genomics 94:117-124.

Hannafon BN, Trigoso YD, Calloway CL, Zhao YD, Lum DH, Welm AL, Zhao ZJ, Blick KE, Dooley WC and Ding WQ (2016) Plasma exosome microRNAs are indicative of breast cancer. Breast Cancer Res 18:90.

Harahap WA, Sudji IR and Nindrea RD (2018) BRCA1 promoter methylation and clinicopathological characteristics in sporadic breast cancer patients in Indonesia. Asian Pac J Cancer Prev 19:2643-2649.

Harman RM, Curtis TM, Argyle DJ, Coonrod SA and Van de Walle GR (2016) A comparative study on the in vitro effects of the DNA methyltransferase inhibitor 5-azacytidine (5-AzaC) in breast/mammary cancer of different mammalian species. J Mammary Gland Biol Neoplasia 21:51-66.

Heishima K, Ichikawa Y, Yoshida K, Iwasaki R, Sakai H, Nakagawa T, Tanaka Y, Hoshino Y, Okamura Y, Murakami M *et al.* (2017) Circulating microRNA-214 and -126 as potential biomarkers for canine neoplastic disease. Sci Rep 7:2301.

Hill MD, Quesnelle C, Tokarski J, Fang H, Fanslau C, Haarhoff Z, Kramer M, Madari S, Wiebesiek A, Morrison J *et al.* (2021) Development of BET inhibitors as potential treatments for cancer: A new carboline chemotype. Bioorg Med Chem Lett 51:128376.

Hillyar C, Rallis KS and Varghese J (2020) Advances in epigenetic cancer therapeutics. Cureus 12:e11725.

Ilango S, Paital B, Jayachandran P, Padma RP and Nirmaladevi R (2020) Epigenetic alterations in cancer. Front Biosci (Landmark Ed) 25:4847.

Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M and Weksberg R (2013) Basic concepts of epigenetics. Fertil Steril 99:607-615.

Jain M, Ingole SD, Deshmukh RS, Bharucha SV, Nagvekar AS, Gaikwad RV and Kharde SD (2021) CEA, CA 15-3, and miRNA expression as potential biomarkers in canine mammary tumors. Chromosom Res 29:175-188.

Jeong S-J, Lee K-H, Nam A-R and Cho J-Y (2019) Genome-wide methylation profiling in canine mammary tumor reveals miRNA candidates associated with human breast cancer. Cancers (Basel) 11:1466.

Jeziorska DM, Murray RJS, De Gobbi M, Gaentzsch R, Garrick D, Ayyub H, Chen T, Li E, Telenius J, Lynch M *et al.* (2017) DNA methylation of intragenic CpG islands depends on their transcriptional activity during differentiation and disease. Proc Natl Acad Sci U S A 114:E7526–E7535.

Jung E-J, Kim I-S, Lee EY, Kang J-E, Lee S-M, Kim DC, Kim J-Y and Park S-T (2013) Comparison of methylation profiling in cancerous and their corresponding normal tissues from Korean patients with breast cancer. Ann Lab Med 33:431-440.

Khandekar D and Tiriveedhi V (2020) Role of BET inhibitors in triple negative breast cancers. Cancers (Basel) 12:784.

Kirn V, Strake L, Thangarajah F, Richters L, Eischeid H, Koitzsch U, Odenthal M and Fries J (2018) ESR1-promoter-methylation status in primary breast cancer and its corresponding metastases. Clin Exp Metastasis 35:707-712.

Kolenda T, Guglas K, Kopczyńska M, Sobocińska J, Teresiak A, Bliźniak R and Lamperska K (2020) Good or not good: Role of miR-18a in cancer biology. Rep Pract Oncol Radiother 25:808-819.

Lee K-H, Shin T-J, Kim W-H and Cho J-Y (2019) Methylation of LINE-1 in cell-free DNA serves as a liquid biopsy biomarker for human breast cancers and dog mammary tumors. Sci Rep 9:175.

Liu D, Xiong H, Ellis AE, Northrup NC, Rodriguez Jr CO, O'Regan RM, Dalton S and Zhao S (2014) Molecular homology and difference between spontaneous canine mammary cancer and human breast cancer. Cancer Res 74:5045-5056.

Loh H-Y, Norman BP, Lai K-S, Rahman NMANA, Alitheen NBM and Osman MA (2019) The regulatory role of MicroRNAs in breast cancer. Int J Mol Sci 20:4940.

López J, Añazco-Guenkova AM, Monteagudo-García Ó and Blanco S (2022) Epigenetic and epitranscriptomic control in prostate cancer. Genes (Basel) 13:378.

Lu B, Wu J, Chen H, Li S and Jia K (2022) LncRNA expression profiles in canine mammary tumors identify lnc34977 as a promoter of proliferation, migration and invasion of canine mammary tumor cells. Vet Sci 9:82.

Markkanen E (2019) Know thy model: Charting molecular homology in stromal reprogramming between canine and human mammary tumors. Front Cell Dev Biol 7:348.

Mohammed SI, Utturkar S, Lee M, Yang HH, Cui Z, Lanman NA, Zhang G, Cardona XER, Mittal SK and Miller MA (2020) Ductal carcinoma in situ progression in dog model of breast cancer. Cancers (Basel) 12:418.

Nam A-R, Lee K-H, Hwang H-J, Schabort JJ, An J-H, Won S-H and Cho J-Y (2020) Alternative methylation of intron motifs is associated with cancer-related gene expression in both canine mammary tumor and human breast cancer. Clin Epigenetics 12:110.

Naz F, Tariq I, Ali S, Somaida A, Preis E and Bakowsky U (2021) The role of long non-coding RNAs (lncRNAs) in female oriented cancers. Cancers (Basel) 13:6102.

Nebbioso A, Tambaro FP, Dell'Aversana C and Altucci L (2018) Cancer epigenetics: Moving forward. PLoS Genet 14:e1007362.

Neganova ME, Klochkov SG, Aleksandrova YR and Aliev G (2022) Histone modifications in epigenetic regulation of cancer: Perspectives and achieved progress. Semin Cancer Biol 83:452-471.

Nicoglou A and Merlin F (2017) Epigenetics: A way to bridge the gap between biological fields. Stud Hist Philos Biol Biomed Sci 66:73-82.

Nishiyama A and Nakanishi M (2021) Navigating the DNA methylation landscape of cancer. Trends Genet 37:1012-1027.

Nurzadeh M, Naemi M and Sheikh Hasani S (2021) A comprehensive review on oncogenic miRNAs in breast cancer. J Genet 100:15.

Osaki T, Sunden Y, Sugiyama A, Azuma K, Murahata Y, Tsuka T, Ito N, Imagawa T and Okamoto Y (2016) Establishment of a canine mammary gland tumor cell line and characterization of its miRNA expression. J Vet Sci 17:385-390.

Pasquini G and Kunej T (2019) A map of the microRNA regulatory networks identified by experimentally validated microRNA-target interactions in five domestic animals: Cattle, pig, sheep, dog, and chicken. OMICS 23:448-456.

Qi M and Xiong X (2018) Promoter hypermethylation of RARb2, DAPK, hMLH1, p14, and p15 is associated with progression of breast cancer: A PRISMA-compliant meta-analysis. Medicine (Baltimore) 97:e13666.

Qiu H and Lind D (2016) Roles of DNA mutation in the coding region and DNA methylation in the 5' flanking region of *BRCA1* in canine mammary tumors. J Vet Med Sci 78:943-949.

Ramadan ES, Salem NY, Emam IA, AbdElKader NA, Farghali HA and Khattab MS (2021) MicroRNA-21 expression, serum tumor markers, and immunohistochemistry in canine mammary tumors. Vet Res Commun 46:377-388.

Raposo LR, Roma-Rodrigues C, Faísca P, Alves M, Henriques J, Carvalheiro MC, Corvo ML, Baptista PV, Pombeiro AJ and Fernandes AR (2017) Immortalization and characterization of a new canine mammary tumour cell line FR37-CMT. Vet Comp Oncol 15:952-967.

Ren X, Li H, Song X, Wu Y and Liu Y (2018) 5-azacytidine treatment induces demethylation of DAPK1 and MGMT genes and inhibits growth in canine mammary gland tumor cells. Onco Targets Ther 11:2805-2813.

Rybicka A, Mucha J, Majchrzak K, Taciak B, Hellmen E, Motyl T and Krol M (2015) Analysis of microRNA expression in canine mammary cancer stem-like cells indicates epigenetic regulation of transforming growth factor-beta signaling. J Physiol Pharmacol 66:29-37.

Sahni JM and Keri RA (2018) Targeting bromodomain and extraterminal proteins in breast cancer. Pharmacol Res 129:156-176.

Schabort JJ, Nam A-R, Lee K-H, Kim SW, Lee JE and Cho J-Y (2020) Ank2 hypermethylation in canine mammary tumors and human breast cancer. Int J Mol Sci 21:8697.

Sharda A, Rashid M, Shah SG, Sharma AK, Singh SR, Gera P, Chilkapati MK and Gupta S (2020) Elevated HDAC activity and altered histone phospho-acetylation confer acquired radio-resistant phenotype to breast cancer cells. Clin Epigenetics 12:4.

Skvortsova K, Stirzaker C and Taberlay P (2019) The DNA methylation landscape in cancer. Essays Biochem 63:797-811.

Smolarz B, Zadrożna-nowak A and Romanowicz H (2021) The role of lncRNA in the development of tumors, including breast cancer. Int J Mol Sci 22:8427.

Sun Y-S, Zhao Z, Yang Z-N, Xu F, Lu H-J, Zhu Z-Y, Shi W, Jiang J, Yao P-P and Zhu H-P (2017) Risk factors and preventions of breast cancer. Int J Biol Sci 13:1387-1397.

Uckelmann M and Davidovich C (2021) Not just a writer: PRC2 as a chromatin reader. Biochem Soc Trans 49:1159-1170.

Vinothini G, Balachandran C and Nagini S (2009) Evaluation of molecular markers in canine mammary tumors: Correlation with histological grading. Oncol Res 18:193-201.

von Deetzen M-C, Schmeck BT, Gruber AD and Klopfleisch R (2014) Malignancy associated MicroRNA expression changes in canine mammary cancer of different malignancies. ISRN Vet Sci 2014:148597.

Vu TL, Nguyen TT, Doan VTH and Vo LTT (2018) Methylation profiles of BRCA1, RASSF1A and GSTP1 in Vietnamese women with breast cancer. Asian Pac J Cancer Prev 19:1887-1893.

Wang C, Zhou Z, Subhramanyam CS, Cao Q, Heng ZSL, Liu W, Fu X and Hu Q (2020) SRPK1 acetylation modulates alternative splicing to regulate cisplatin resistance in breast cancer cells. Commun Biol 3:268.

Xavier PLP, Cordeiro YG, Alexandre PA, Pires PRL, Saranholi BH, Silva ER, Müller S and Fukumasu H (2019) An epigenetic screening determines BET proteins as targets to suppress self-renewal and tumorigenicity in canine mammary cancer cells. Sci Rep 9:17363.

Xi Y, Shi J, Li W, Tanaka K, Allton KL, Richardson D, Li J, Franco HL, Nagari A, Malladi VS *et al.* (2018) Histone modification profiling in breast cancer cell lines highlights commonalities and differences among subtypes. BMC Genomics 19:150.

Xie Y, Shi Z, Qian Y, Jiang C, Liu W, Liu B and Jiang B (2022) HDAC2- and EZH2-mediated histone modifications induce PDK1 expression through miR-148a downregulation in breast cancer progression and adriamycin resistance. Cancers (Basel) 14:3600.

Xu E, Hu M, Ge R, Tong D, Fan Y, Ren X and Liu Y (2021) LncRNA-42060 regulates tamoxifen sensitivity and tumor development via regulating the miR-204-5p/SOX4 axis in canine mammary gland tumor cells. Front Vet Sci 8:654694.

Yadav P, Masroor M, Nandi K, Kaza RCM, Jain SK, Khurana N and Saxena A (2018) Promoter methylation of BRCA1, DAPK1 and RASSF1A is associated with increased mortality among Indian women with breast cancer. Asian Pac J Cancer Prev 19:443-448.

Yari H, Shabani S, Nafissi N, Majidzadeh T and Mahjoubi F (2022) Investigation of promoter methylation patterns association with genes expression profile of ISL1, MGMT and DMNT3b in tissue of breast cancer patients. Mol Biol Rep 49:847-857.

Zhang L, Qu J, Qi Y, Duan Y, Huang Y-W, Zhou Z, Li P, Yao J, Huang B, Zhang S *et al.* (2022) EZH2 engages TGFβ signaling to promote breast cancer bone metastasis via integrin β1-FAK activation. Nat Commun 13:2543.

Zhang T, Cooper S and Brockdorff N (2015) The interplay of histone modifications – writers that read. EMBO Rep 16:1467-1481.

Zhou W-M, Liu B, Shavandi A, Li L, Song H and Zhang J-Y (2021) Methylation landscape: Targeting writer or eraser to discover anti-cancer drug. Front Pharmacol 12:690057.

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