SciMedCentral

JSM Clinical Case Reports

Research Article

Immunohistochemical Study of MMP-9 Expression in Canine Malignant Mammary Tumors

Elahirad E[1]*, Tavassoly A[1], and Golshahi H[2]

[1]Department of Pathology, University of Tehran, Iran

[2]Nanobiotechnology Research Center, Avicenna Research Institute, Iran

*Corresponding author
Elnaz Elahirad, Department of Pathology, University of Tehran, Iran

Submitted: 26 December, 2023
Accepted: 27 January, 2024
Published: 29 January, 2024

ISSN: 2373-9819

OPEN ACCESS

Keywords
- Dog
- Immunohistochemistry
- MMP-9
- Mammary neoplasms

Abstract

A class of proteinases called matrix metalloproteinases (MMPs) is involved in the breakdown of the extracellular matrix (ECM) as well as other biological activities. Due to their capacity to degrade the matrix, they were considered in the invasion and metastasis of tumors. The primary goal of this study is to evaluate the association between MMP-9 expression in tumor-adjacent stromal cells and malignant mammary tumors in dogs, as well as the potential use of this protease as a predictive tumor marker. During surgery and necropsy, 32 canine malignant mammary gland tumors were discovered. For histopathological grading of tumors, the Pena et al. [1], approach and the Goldsmith et al. [2], method were employed. Utilizing immunohistochemical labeling, epithelial and stromal cell MMP-9 expression was identified. The level of tumor malignancy was associated with the degree of MMP-9 expression in stromal cells. In addition, stromal cells had much greater levels of MMP-9 expression. The expression of MMP-9 was well distributed and intense in all grade II and III carcinomas as well as poorly differentiated carcinosarcomas. High histologic grade tumors have greater levels of MMP-9 expression, according to semiquantitative examination of the gene's expression. This finding lends credence to the concept that MMP-9 functions as an ECM component in tumor invasion and metastasis.

INTRODUCTION

Researchers and veterinarians are paying attention to neoplasia as a major problem. Small animals are now being used as models for research on human cancer, either for the discovery of new biomarkers or innovative treatment approaches, due to the rising incidence and death rates of tumors [3,4]. Neoplasia occurs sporadic and with varying degrees of morbidity in agricultural animals. The latter result in the condemnation of carcasses and/or organs in a herd or flock, which has an economic cost [5]. Studies from various locations have shown varying incidence rates and mortality, which is suggestive of the influence of environmental variables on the development and prognosis of tumors. Malignant canine mammary tumors may be as high as 50% [6]. According to Santos AA. et al.[7], invasion, metastasis, and recurrence of the tumor are the primarcauses of canine mammary tumor mortality.

In human malignancies, tumor invasion and metastasis are predictive of a poor prognosis. Water, minerals, proteoglycans, and fibrous proteins including collagen, elastin, and laminin make up the extracellular matrix (ECM), a dynamic non-cellular structure [8-10]. Proteinases secreted by residing cells continuously alter its constituent parts. Research on human breast cancer has revealed that the interaction between tumor cells and stromal cells promotes angiogenesis, invasion, and proliferation by triggering matrix-related proteases including MMPs and serine proteases [10-13]. The extracellular matrix (ECM) is degraded by endopeptidases known as MMPs, which include collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and additional MMPs. They take part in a variety of biochemical processes that affect a variety of physiological and pathological states, including tissue remodeling, angiogenesis, ovulation, wound healing, and bone resorption [10,14]. In addition to T cells, neutrophils, monocytes, macrophages, leukocytes, endothelium cells, breast epithelial cells, osteoclasts, and keratinocytes, a variety of other cells also manufacture MMP-9, a gelatinase [15,16]. MMP-9 is produced in a dormant or latent state and released as a proenzyme or zymogen. According to Danielle et al. [17], Kessenbrock [8], and Bauvois [9], it manifests as a monomer in plasma and a dimer in neutrophils. MMP-9 is implicated in a number of neoplastic development pathways, including angiogenesis, invasion, and metastasis, according to accumulating data [11,18]. Immunohistochemistry is a method used to detect particular macromolecules connected to certain biological systems [19]. Experimental evidence implies MMP-9 expression in various canine neoplastic tissue including skin, bone, and mammary gland [7,20,21]. The present work aims to evaluate the relationship between MMP-9 expression and tumor histologic type and grade in epithelial and stromal cells of canine mammary cancers.

MATERIAL AND METHODS

Ethical statement

We utilized animals for the study according to the US National

Institute of Health, Policy on Humane Care and Use of Laboratory Animals (PHS). This manuscript is author's original work and has not been published elsewhere. This manuscript is authors' original work and has not been published elsewhere. We delivered analysis appropriate to the context in which the research is held.

Sample collection and histopathology

During surgery or necropsy, 32 canine mammary tumors were found. Samples were cut into 5μ thick slices and stained with H&E after being fixed in 10% buffered formalin. The tissue that was selected for the immunohistochemistry analysis was nearby. Two impartial observers assessed the histopathological types of tumors in accordance with the methodology of Goldsmith et al. [2]. Tubule formation, nuclear pleomorphism, and mitoses per HPF are the three key characteristics used in Pna et al.'s classification of tumors.

Immunohistochemistry

On each sample, immunohistochemical staining was done. Utilizing anti-goat monoclonal antibodies from rabbits that have been biotinized, immunohistochemical staining was carried out. Formalin-fixed, paraffin-embedded tissue slices that were cut at a 3μ m thickness were used for the reaction. Antigen retrieval was done using a microwave oven at 750 W and 180 W (citrate buffer, 15 minutes, PH6, 0/01M) after deparaffinization and rehydration. To neutralize endogenous peroxidase, all transparencies were incubated with 3% hydrogen peroxide for five minutes. To reduce nonspecific binding, all transparencies were incubated with rabbit and mouse serum for 20 minutes. The transparencies were incubated with the primary antibody (goat polyclonal antibody anti-MMP-9 diluted 1 to 200) for several hours. The transparencies were then incubated with a secondary antibody (rabbit anti-goat biotinized antibody diluted 1 to 100) for 30 minutes. In this phase, the transparencies were incubated for 10 minutes in a solution of diaminobenzidine (substrate) and chromogen (1 drop of chromogen per 1 ml of substrate). The specimens were then counterstained with hematoxylin. As a positive control, blood vessel muscle was used, while non-immunogenic goat immunoglobulin was substituted for the primary antibody as a negative control. Evaluation of immunostained sections in a semiquantitative manner. Two observers estimated the proportion of stromal and malignant cells with cytoplasmic staining. Eventually, tumors classified as low (< 50%) or high (≥ 50%) expression level for stromal cells and low (< 25%) or high (≥ 25%) expression level for epithelial cells Table 1.

Statistical analysis

To analyze differences of MMP-9 expression in stromal and epithelial cell, the fisher exact test was used and the pearson $\varkappa^2$ test was used to analyze the correlation of MMP-9 expression and histologic grade of the tumors. Statistical analysis performed using Graphpad-Prism version 9 (Graphpad software, San Diego, CA, USA). A p-value of < 0.05 was considered statistically significant.

Table 1: MMP-9 expression in canine malignant mammary tumors

Histologic classification	MMP-9 extension		MMP-9 intensity	
	Neoplastic cells	Stromal cells	Neoplastic cells	Stromal cells
Tubular carcinoma – Grade I	Low	Low	0	0
Tubular carcinoma – Grade I	Low	Low	0	0
Tubular carcinoma – Grade II	Low	High	1+	2+
Tubular carcinoma – Grade II	Low	Low	1+	1+
Tubular carcinoma – Grade II	Low	High	1+	2+
Tubulopapillary carcinoma – Grade II	Low	High	0	3+
Tubulopapillary carcinoma – Grade I	Low	High	0	1+
Tubulopapillary carcinoma - Grade I	Low	High	0	2+
Tubulopapillary carcinoma – Grade II	High	High	2+	2+
Cystic papillary carcinoma – Grade III	Low	High	1+	3+
Cystic papillary carcinoma – Grade II	Low	High	0	2+
Cystic papillary carcinoma – Grade II	Low	High	0	2+
Cystic papillary carcinoma – Grade II	Low	High	0	2+
Cribriform carcinoma – Grade III	High	High	2+	3+
Cribriform carcinoma – Grade II	Low	Low	0	2+
Solid carcinoma – Grade III	Low	High	0	3+
Comedocarcinoma – Grade III	High	Low	2+	0
Complex carcinoma – Grade II	High	High	1+	2+
Complex carcinoma – Grade II	Low	High	1+	2+
Carcinoma and malignant myoepithelioma – Grade II	Low	High	0	2+
Carcinoma-mixed type – Grade I	Low	Low	0	1+
Carcinoma-mixed type – Grade I	Low	Low	0	1+
Carcinoma-mixed type – Grade II	Low	Low	1+	2+
Carcinoma-spindle cell variant – Grade III	Low	High	0	3+
Carcinoma-spindle cell variant – Grade II	Low	High	1+	3+
Carcinoma-spindle cell variant – Grade II	High	High	1+	3+
Malignant myoepithelioma – Grade II	High	High	2+	3+
Chondrosarcoma	High	High	2+	3+
Carcinosarcoma	Low	High	1+	3+
Carcinosarcoma	Low	High	0	3+
Carcinosarcoma	Low	High	0	3+
Carcinosarcoma	Low	High	0	3+

RESULTS

Malignant breast tumors were determined in all 32 samples. The most numerous neoplasms were simple carcinomas– 15 (46.8%), then carcinosarcoma–4 (12.5%), carcinoma mixed type–3 (9%) and carcinoma–spindle cell variant–3 cases (9%). Detailed histopathological results are presented in Table 1. Six malignant neoplasms were graded as grade I, 16 as grade II, and five as grade III out of 32 total (Figures 1-5). Sparse stroma and a low to moderate mitotic activity index were seen in tubular carcinomas. One tubular carcinoma, one cystic papillary carcinoma, one cribriform carcinoma, and one comedocarcinoma all showed lymphatic invasion. In two instances of tubulopapillary cancer, desmoplasia was seen. Solid carcinoma reported to have extensive necrosis. Results of the histopathologic examination of tumors of the canine mammary gland and the semiquantitative measurement of MMP-9 expression are shown in Table 2. In 21.8% of tumors, epithelial cells had high MMP-9 expression, whereas in 71.8% of tumors, stromal cells had high MMP-9 expression.

In general, stromal cells of all histologic types of neoplasms expressed MMP-9. There was no evidence of MMP-9 expression in the neoplastic epithelial cells of 17 neoplasms, including 2 tubular carcinoma, 3 tubulopapillary carcinoma, 3 cystic papillary carcinoma, 1 cribriform carcinoma, 1 solid carcinoma, 1 carcinoma and malignant epithelioma, 2 carcinoma mixed type, 1 carcinoma-spindle cell variant, and 3 carcinosarcoma. MMP-9 expression was most prominent in neoplasms made up of malignant spindle cells (epithelial or mesenchymal). Neoplasms with intensity ratings of +3 also displayed substantial levels of extension. The histologic type and grade of neoplasms were not linked with MMP-9 expression in neoplastic cells and stromal cells. MMP-9 expression was shown to be most intense and dispersed in grade II and III carcinomas as well as poorly differentiated carcinosarcoma.

DISCUSSION

Both the stroma surrounding tumors and the neoplastic cells are the subject of oncology study. The ECM acts as a natural inhibitor of tumor growth. The stability of tissue proliferation is guaranteed by ECM integrity throughout normal tissue remodeling and degradation. The importance of ECM in neoplastic transformation has been confirmed by an expanding body of research. Invasion of malignant cells into basement membrane and surrounding healthy tissue, influx of neoplastic cells into lymphatics and blood arteries, and metastasis to distant organs are all facilitated by proteolytic enzymes [8,15,22]. According to studies Björklund M [15], Fingleton [23], Biljana E [24], tumor progression necessitates the cleavage and activation of growth factors, proteinases and their inhibitors, blood clotting factors, cell surface receptors, adhesion molecules, and intracellular substrates, followed by the cleavage of extracellular matrix components. Proteases were subsequently presented as useful tumor indicators.

Extracellular proteins are thought to enhance the malignant phenotype by generating proangiogenic and proinflammatory characteristics in the tumor microenvironment. Veterinary studies looked on the many roles that MMP-9 plays in the development of tumors. The first important stage in neoplastic cells' intravasation (vascular invasion) is the degradation of the subendothelial basement membrane. Type IV collagen, a significant component of basement membrane, is degraded by MMP-2 and MMP-9 [11,12]. Through the oxidation of VEGF, bFGF, TGF- β, and type IV collagen, MMP-9 stimulates vasculogenesis. On the other hand, MMP-9 suppresses angiogenesis and endothelial cell proliferation by producing endostatin and angiostatin. Time of expression and substrate availability determine its function in angiogenic and angiostatic pathways [8,18,25,26]. It is believed that neoplastic cells coordinate the production of certain growth factors and cytokines during various phases of carcinogenesis. Following the formation of MMPs like MMP-9, the production of cytokines like TGF-ß (Transforming growth factor-ß), PDGF (Platelet derived growth factor), and ECM metalloproteinase inducer increases [13,27]. It is unclear what function MMP plays in diseases in animals. The normal canine mammary gland expresses MMP-9. The increased expression during pregnancy is a sign that MMP-9 is involved in the interactions between tissues throughout the developmental and proliferative phases. MMP-9 is expressed during the physiological remodeling of the mammary gland, according to research using a mouse model. ECM breakdown and cell proliferation are also involved in tumor development, invasion, and metastasis [28]. Using techniques including zymography, ELISA, western blot, and immunohistochemistry, earlier research found that MMP-9 expression is greater in malignant mammary neoplasms than in benign mammary neoplasms [7,17,29,30]. Jinga et al. [14], reported that invasive carcinomas, demonstrated the highest levels of MMP-9 expression and activity.

MMP-9 has been presented as a distant metastatic predictor in human breast cancer. Studies looked for evidence of stromal cell involvement in the development of neoplasms. Studies have shown that MMP-9 is present in the stromal fibroblasts and endothelial cells surrounding breast in situ and invasive carcinomas [31]. MMP-9 expression in diverse canine mammary tumor histopathologic types was not substantially different in the present investigation. Our results are consistent with those of Gramulia et al. [32], and Santos et al [7]. According to our findings, stromal and neoplastic cells expressed MMP-9 to varying degrees. The histologic type and grade of the tumor did not substantially correlate with its expression. Our findings concur with other studies that argued MMP-9 expression in malignant and stromal cells.

Additionally, immunostained sections examination indicated a widespread MMP-9 expression pattern. However, Gramulia et al. discovered that the enzyme had both a diffuse and granular staining pattern. The level of tumor malignancy was associated with the degree of MMP-9 expression in stromal cells. MMP-9 expression was greater in grade III neoplasms than in grade II and I neoplasms and in metastatic neoplasms than in non-metastatic neoplasms [34]. This finding lends credence to the notion that MMP- 9 is involved in tumor invasion and metastasis.

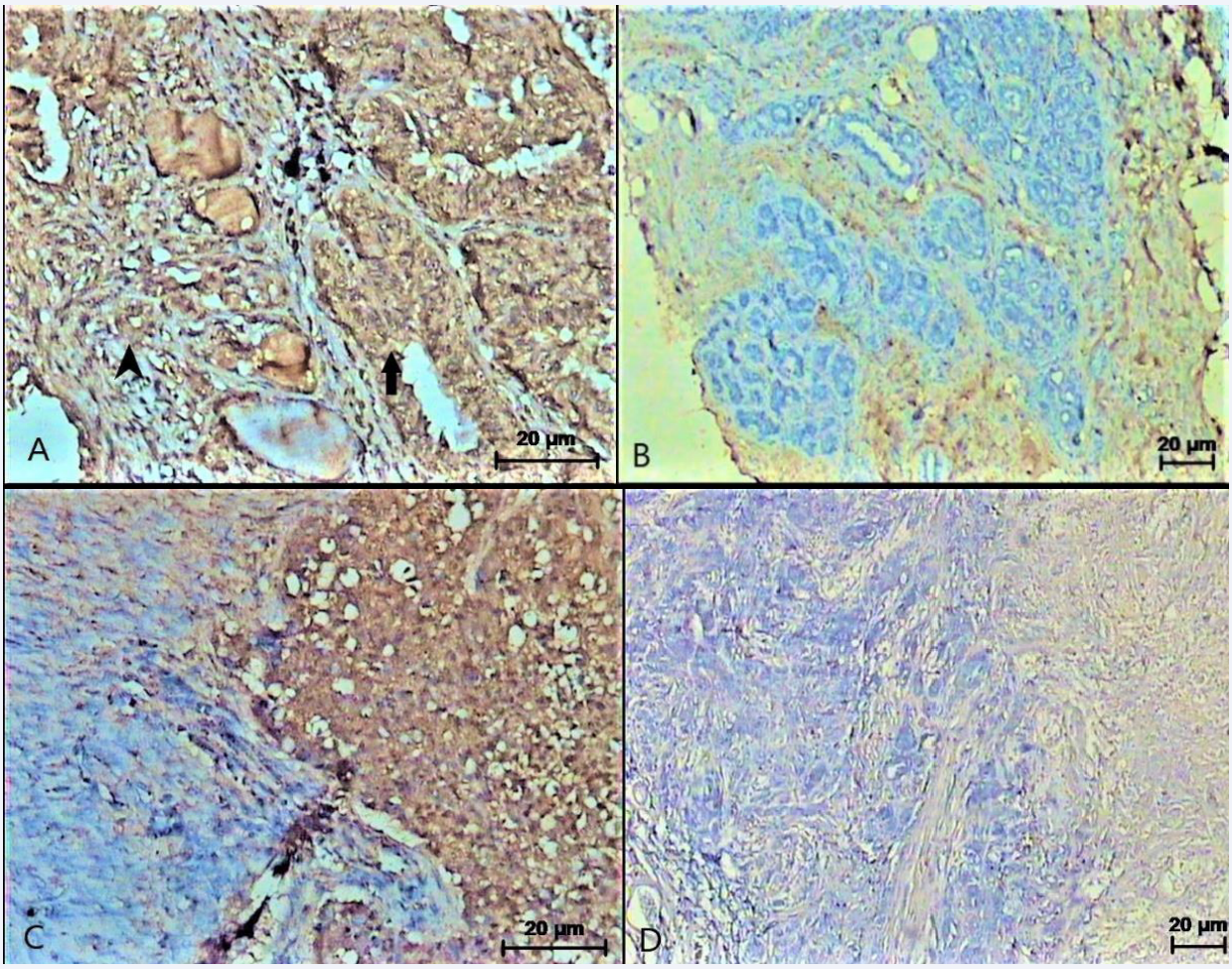

Figure 1 A. Low differentiated carcinosarcoma. High expression of MMP-9 in neoplastic epithelial cells (arrow, brown) and stromal cells (arrowhead, brown). B. Low differentiated carcinosarcoma, expression of MMP-9 by stromal cells (brown) and lack of expression in mammary epithelial cells (blue). C. Comedocarcinoma. High expression of MMP-9 by neoplastic epithelial cells (brown) and lack of expression in stromal cells (blue). D. Carcinoma-spindle cell variant. Lack of expression of MMP-9 in stromal cells and neoplastic epithelial cells (blue).

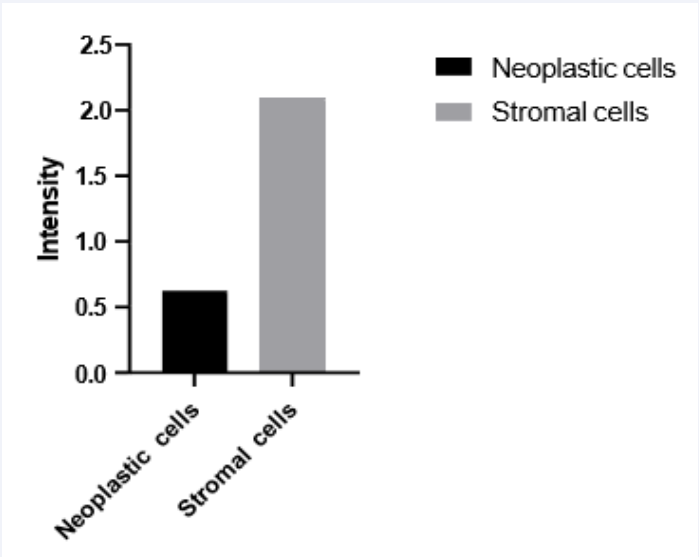

Figure 2 Mean of MMP-9 expression in neoplastic epithelial cells and stromal cells.

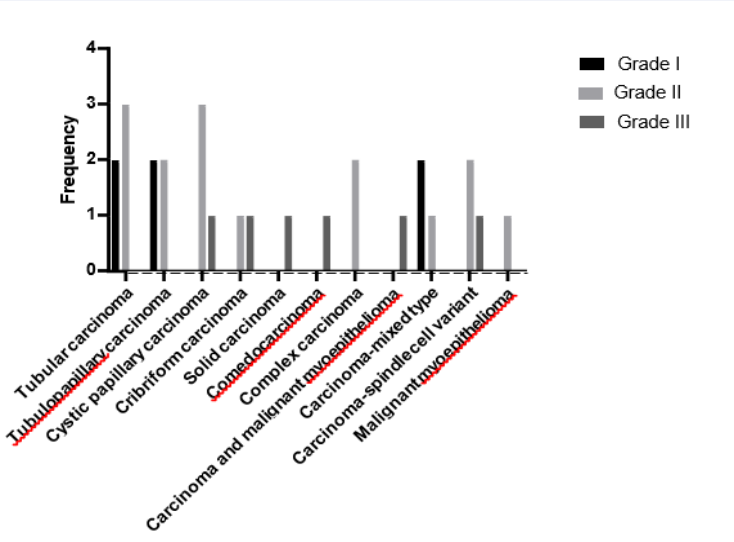

Figure 3 Frequency of histologic type of canine mammary tumors according to histologic grade.

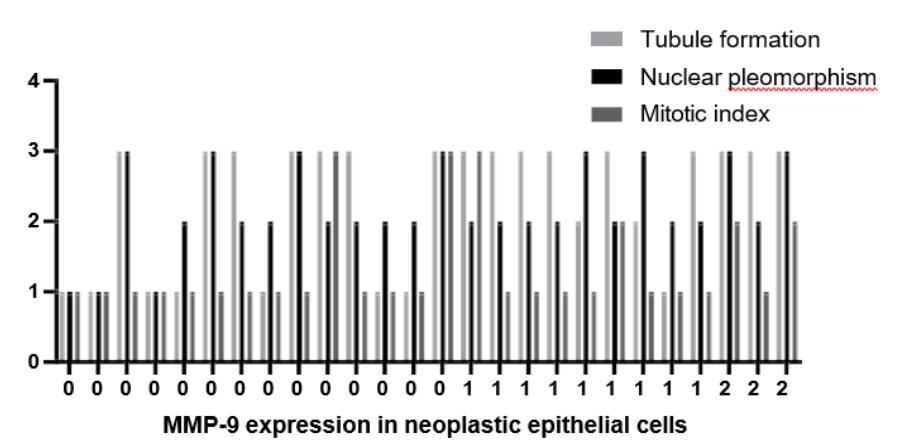

Figure 4 Analysis of correlation of MMP-9 expression in neoplastic epithelial cells and three parameters of histologic grading of mammary tumors are demonstrated. The three parameters were not significantly correlated (P>0.01) with MMP-9 expression.

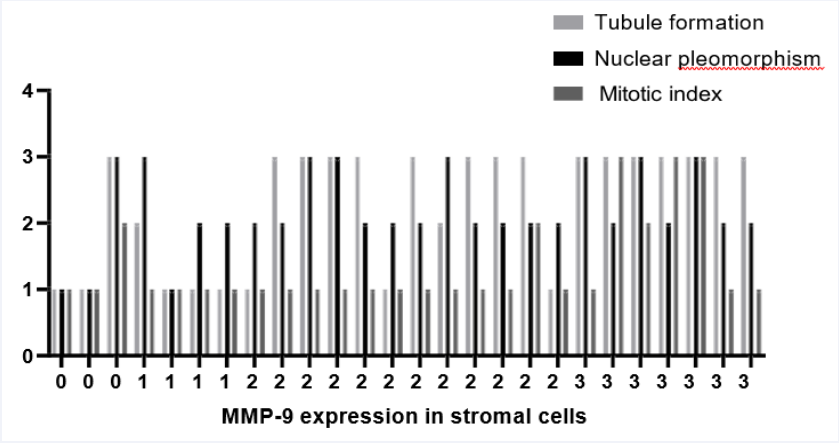

Figure 5 Analysis of correlation of MMP-9 expression in stromal cells and three parameters of histologic grading of mammary tumors are demonstrated. The three parameters were not significantly correlated (P>0.01) with MMP-9 expression in stromal cells.

Table 2: Histopathologic examination of tumors of the canine mammary gland and the semiquantitative measurement of MMP-9 expression.

		A	B	C
Correlation		**MMP-9 expression in stromal cells vs.**	**MMP-9 expression in stromal cells vs.**	**MMP-9 expression in stromal cells vs.**
		Tubule formation	Nuclear pleomorphism	Mitotic index
1	**Pearson r**			
2	r	0.585	0.3698	0.368
3	95% confidence interval	0.2556 to 0.7927	-0.02046 to 0.6623	-0.02255 to 0.6611
4	R squared	0.3423	0.1368	0.1354
5				
6	**P value**			
7	P (two-tailed)	0.0017	0.063	0.0644
8	P value summary	**	ns	ns
9	Significant? (alpha = 0.05)	Yes	No	No
10				
11	**Number of XY Pairs**	26	26	26

In veterinary medicine, MMP-9 was found in canine cutaneous mast cell tumors, melanomas, osteosarcomas, and mammary neoplasms. Previous research on MMP-9 expression in canine mammary gland cancers was conducted by Nowak et al. [34], Santos et al. [7], Chen et al. [35], and Dong et al [36]. According to the present research, tubule development is connected with MMP-9 expression in epithelial cells and stromal cells, with P values of 0.0479 for cancerous cells and 0.017 for stromal cells, respectively. MMP-9 expression was found in the cytoplasm and nucleus of canine mammary neoplastic cells, according to another research by Karakurt et al [30]. Mendes et al. [37], however, were unable to find MMP-9 in brain samples. They claimed that the MMP-9 levels in the samples were below the technique's detection thresholds. Uncertainty surrounds the location of MMP production [37]. MMP-9 is a protease that is generated in locations other than neoplastic and stromal cells, according to several researchers who disagreed regarding the location of its synthesis [27]. MMP-9 is physiologically active in the tumor microenvironment. Shia et al. noted that MMP-9 activity in tumor tissue is connected with serum level of MMP-9 in addition to its function in angiogenesis. Research studies that shown col1a1, col3a1, col4a1, and col5a1 is connected with poor prognosis of gastric cancer and glioblastoma Chen YC [35], Jiang [38], Henke [39], provide the clearest evidence of the significance of collagenolytic activity of MMP-9. There is debate concerning the relationship between MMP-9 expression and the histologic grade of tumors. The argument that many signaling pathways are connected via MMP-9 bioactivity might be used to refute contrary results. Additionally, there are a variety of effects when MMP-9 activity is increased or decreased. When taken as a whole, an examination of the materials notes that the primary significance of MMPs in the invasion of neoplasms is related to their extensive proteolytic capacities. Their capacity to catalyze the ECM results in the cleavage and destruction of ECM proteins. Additionally, they validated their diagnostic and prognostic values [17,29,33]. The key finding is that our study supports prior hypotheses on MMP-9 expression by stromal and epithelial cells in canine mammary gland cancers [40].

Highlights

- Matrix metalloproteinases are one of the ECM components which have proteolytic activity.
- MMP-9 is a member of MMPs family which is activated in in variety of physiologic processes. Further, it is detected in cancerous tissue, including breast, skin, and bone.
- MMP-9 is produced by diverse populations of inflammatory, epithelial, endothelial, and stromal cells.
- The zinc containing protease is involved in process of tumor invasion and metastasis.

REFERENCES

1. Peña L, De Andrés PJ, Clemente M, Cuesta P, Pérez-Alenza MD. Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics. Vet Pathol. 2013; 50: 94-105.
2. Goldschmidt M, Peña L, Rasotto R, Zappulli V. Classification and Grading of Canine Mammary Tumors. Vet Pathol. 2011; 48: 117-131.
3. Abdelmegeed SM, Mohammed S. Canine mammary tumors as a model for human disease. Oncol Lett. 2018; 15: 8195-8205.
4. 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; 10: 617.
5. Schlafer DH, Foster RA. Femal genital system. Jubb, Kennedy & Palmer's Pathology of Domestic Animals. 2016; 3: 358-464.
6. Garcia APV, Reis LA, Nunes FC, Longford FGJ, Frey JG, de Paula AM, et al. Canine mammary cancer tumour behaviour and patient survival time are associated with collagen fibre characteristics. Sci Rep. 2021; 11: 5668.
7. Santos AA, Lopes CC, Marques RM, Amorim IF, Gärtner MF, de Matos AJ. Matrix metalloproteinase-9 expression in mammary gland tumors in dogs and its relationship with prognostic factors and patient outcome. Am J Vet Res. 2012; 73: 689-697.
8. Kessenbrock K, Plaks V, Plaks V. Matrix Metalloproteinases: Regulators of the Tumor Microenvironment. Cell. 2010; 141: 52-67.
9. Bauvois B. New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: Outside-in signaling and relationship to tumor progression. Biochim Biophys Acta. 2012; 1825: 29-36.
10. Walker C, Mojares E, Del Río Hernández A. Role of extracellular matrix in development and cancer progression. Int J Mol Sci. 2018; 19: 3028.
11. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Itoh T, et al. Matrix metalloproteinase-9 triggers theangiogenic switch during carcinogenesis. Nat Cell Biol. 2000; 2: 737-744.
12. Deryugina EI, Deryugina EI. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev. 2006; 25: 9-34.
13. Perentes Y, Kirkpatrick ND, Kirkpatrick ND, Smith EY, Shaver CM, Sgroi D, et al. Cancer Cell–Associated MT1-MMP Promotes Blood Vessel Invasion and Distant Metastasis in Triple-Negative Mammary Tumors. Cancer Res. 2011; 71: 4527-4538.

14. Jinga DC, Blidaru A, Condrea I, Ardeleanu C, Dragomir C, Szegli G, et al. MMP-9 and MMP-2 gelatinases andTIMP-1 and TIMP-2 inhibitors in breast cancer: correlations with prognostic factors. J Cell Mol Med. 2006; 10: 499-510.

15. Björklund M, Koivunen E. Gelatinase-mediated migration and invasion of cancer cells. Biochim Biophys Acta. 2005; 1755: 37-69.

16. Tonti GA, Mannello F, Cacci E, Biagioni S. Neural stem cells at the crossroads: MMPs may tell the way. Int J Dev Biol. 2009; 53: 1-17.

17. Daniele A, Zito AF, Giannelli G, Divella R, Asselti M, Mazzocca A, et al. Expression of Metalloproteinases MMP-2 andMMP-9 in Sentinel Lymph Node and Serum of Patients with Metastatic and Non-Metastatic Breast Cancer. Anticancer Res. 2010; 30: 3521-3528.

18. Handsley MM, Edwards DR. Metalloproteinases and their inhibitors in tumor angiogenesis. Int J Cancer. 2005; 115: 849-860.

19. de Matos LL, Trufelli DC, Trufelli DC, da Silva Pinhal MA. Immunohistochemistry as an Important Tool in Biomarkers Detection and Clinical Practice. Biomark insights. 2010: 5: 9-20.

20. Beekman DJ, Zandvliet MMJM, Keesler RI. Prognostic Factors for Canine for canine cutaneous mast cell tumors including Ki67, MAC387, Factor 8 and MMP-9 immunohistochemistry. Biology. 2014.

21. Gieger TL, Nettifee-Osborne J, Hallman B, Johannes C, Clarke D, Nolan MW, et al. The impact of carboplatin and toceranib phosphate on serum vascular endothelial growth factor (VEGF) and metalloproteinase-9 (MMP-9) levels and survival in canine osteosarcoma. Can J Veter Res. 2017; 81: 199-205.

22. Mannello F, Medda V. Nuclear localization of Matrix metalloproteinases. Prog Histochem Cytochem. 2012; 47: 27-58.

23. Fingleton B. Matrix metalloproteinases as valid clinical targets. Curr pharm Des. 2007; 13: 333-346.

24. Biljana E, Velickovski B, Cena D, Daniela V. Matrix metalloproteinases (with accent to collagenases). J Cell Animal Biol. 2011; 5: 113-120.

25. Foda HD, Zucker S. Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. Drug Discov Today. 2001; 6: 478-482.

26. López-Otín C, Palavalli LH, Samuels Y. Protective roles of matrix metalloproteinases: From mouse models to human cancer. Cell Cycle. 2009; 8: 3657-3662.

27. Quaranti M, Daniele A, Coviello M, Venneri MT, Abbate I, Caringella ME, et al. MMP-2, MMP-9, VEGF and CA 15.3 in Breast Cancer. Anticancer Res. 2007; 27: 3593-3600.

28. Pupaki DV, Ankova D, Vasilev VP, Rashev PI. Localization of MMP-2 and MMP-9 in canine mammary gland. J Biomed Clin Res. 2014; 7: 99-104.

29. Lana SE, Ogilvie GK, Hansen RA, Powers BE, Dernell WS, Withrow SJ. Identification of matrix metalloproteinases in canine neoplastic tissue. Am J Vet Res. 2000; 61: 111-114.

30. Karakurt E, Kuru M, DAG S, Beytut E, Oral H, Nuhoglu H, et alImmunohistochemically Evaluation of PCNA and MMP-9 Expressions in Different Types of Canine mammary carcinomas. Dicle Üniversitesi Veteriner Fakültesi Dergisi. 2020; 13: 119-124.

31. Behrens P, Rothe M, Wellmann A, Krischler J, Wernert N. The Ets-1 transcription factor is up-regulated togetherwith MMP 1 and MMP 9 in the stroma of pre-invasive breast cancer. J Pathology. 2001; 194: 43-50.

32. Gramulia BC, Pulz LH, Mota EFF, Fukumasu H, Strefezzi RF. Matrix metalloproteinase 9 expression in canine mammary carcinomas. Braz J Vet Pathol. 2016; 9: 55-61.

33. Vinothini G, Balachandran C, Nagini S. Evaluation of Molecular Markers in Canine Mammary Tumors: Correlation with Histological Grading. Oncol Res. 2009; 18: 193-201.

34. Nowak M, Madej JA, Podhorska-Okolow M, Dziegiel P. Expression of Extracellular Matrix Metalloproteinase (MMP-9), E-Cadherin and Proliferation-associated Antigen Ki-67 and their Reciprocal Correlation in Canine Mammary Adenocarcinomas. In Vivo. 2008; 22: 463-469.

35. Chen YC, Chang SC, Huang YH, Lee YJ, Chang CC, Liao JW, et al. Expression and the Molecular Forms of Neutrophil Gelatinase-associated Lipocalin and Matrix Metalloproteinase 9 in Canine Mammary Tumors. Vet Comp Oncol. 2019; 17: 427-438.

36. Dong H, Diao H, Zhao Y, Xu H, Pei S, Gao J, et al. Overexpression of Matrix Metalloproteinase-9 in Breast Cancer Cell Lines Remarkably Increases the Cell Malignancy Largely via Activation of Transforming Growth Factor Beta/SMAD Signalling. Cell Prolif. 2019; 52: e12633.

37. Mendes O, Kim HT, Stoica G. Expression of MMP2, MMP9 and MMP3 in breast cancer brain metastasis in a rat model. Clin Exp Metastasis. 2005; 22: 237-246.

38. Jiang YZ, Ma D, Suo C, Shi J, Xue M, Hu X, et al. Genomic and transcriptomic landscape of triple-negative breast cancers: subtypes and treatment strategies. Cancer cell. 2019; 35: 428-440.

39. Henke E, Nandigama R, Ergün S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. Frontiers Mol Biosci. 2020; 6: 160.

40. Loukopoulos P, Mungall BA, Robinson WF, Straw RC, Thornton JR, Matrix metalloproteinase-2 and -9 involvement in canine tumors. Veterinary Pathology. 2003; 40: 382-394.