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Early View

Original Research Article

MMP-9 Immunoexpression in Canine Cutaneous Squamous Cell Carcinomas: A Preliminary Study

Key words

dog;
immunohistochemistry;
metalloproteinase-9;
squamous cell carcinoma

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Abstract: Cutaneous squamous cell carcinoma (SCC) is the second most common type of skin cancer in humans and one of the most frequent in dogs. Interest in studying this tumor type in animals has grown significantly, largely due to its high mutational load. Among the biomarkers, metalloproteinases (MMPs) have stood out. MMPs are a family of calcium- and zinc-dependent proteases that degrade the extracellular matrix (ECM), with MMP-9, a gelatinase, among the most investigated. MMP-9 expression has been reported to be increased in several tumors. The main objective of this study was to evaluate MMP-9 immunostaining in canine SCC. A descriptive analysis of MMP-9 expression was performed, categorizing it in tumor cells as absent, weak, moderate, or strong. In the SCC cases studied, with respect to marking intensity, 27 tumors showed strong intensity (+++), 6 moderate intensity (++), and 4 weak intensity (+). In all degrees of differentiation, a strong staining intensity predominated. In addition, we found MMP-9 expression in the stroma and macrophages, suggesting its role in tumor progression and in modulating the tumor microenvironment. These findings highlight the potential of MMP-9 as a prognostic biomarker. Understanding its role in SCC could significantly improve our ability to predict disease progression in dogs, leading to more effective treatment strategies.

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Introduction

Cutaneous squamous cell carcinoma is among the most frequently encountered malignant skin tumors in dogs (1). It is characterized by locally aggressive and infiltrative behavior, while distant metastasis is generally uncommon, except in digital forms, which show a higher risk of regional and distant spread. Cutaneous SCC may cause marked local tissue destruction, pain, and functional impairment, often requiring wide or radical surgical excision, and in some advanced or anatomically challenging cases may become non-resectable, leading to consideration of euthanasia on welfare grounds (2–4).

Cutaneous SCC occurs most frequently in medium- to large-breed dogs between 6 and 13 years of age, with no sex predisposition (5). Breeds such as Beagle, Pit Bull, Schnauzer, Basset Hound, Collie, and Dalmatian appear to be overrepresented. Several factors contribute to its development, including chronic ultraviolet radiation exposure, persistent inflammatory processes, immunosuppression, viral infections, repetitive trauma, epidermal pigment deficiency, and skin burns (6–8).

Classically, the diagnosis of cutaneous SCC and other cutaneous tumors in dogs relies on histopathological examination of biopsy or surgical excision specimens, which remains the gold standard for definitive tumor classification, grading, and staging. In clinical practice, this approach is often preceded by fine-needle aspiration, a rapid and minimally invasive technique that provides a preliminary cytological assessment and supports the suspicion of malignancy (9–11). However, this technique does not provide information on tissue architecture or allow identification of the specific tumor type, limiting its ability to fully classify the neoplasm, and it does not replace histopathology for definitive diagnosis (9–14).

Despite the availability of these diagnostic approaches, advanced SCC often responds poorly to treatment and exhibits a high mutational burden, suggesting a challenging biological behavior (15–17). Therefore, identifying complementary biomarkers that aid diagnosis, prognosis, and understanding of tumor progression is crucial (15).

Among the molecular targets of interest, matrix metalloproteinases (MMPs) play crucial roles in tumor progression, acting both directly by degrading the extracellular matrix and basement membrane, favoring

the proliferation and dissemination of neoplastic cells, and indirectly by stimulating angiogenesis, which is fundamental for nutrient supply and tumor expansion. Increased expression of these enzymes is associated with more invasive and metastatic phenotypes, while their role in reorganizing extracellular matrix components facilitates cell migration through the tumor microenvironment (18–20).

MMP-9 is one of the most studied metalloproteinases. Produced by various cells, including keratinocytes, monocytes, macrophages, and polymorphonuclear leukocytes, it regulates extracellular matrix degradation and cleaves gelatin and collagen types IV, V, XI, and XVI, processes essential for invasion and metastasis. Furthermore, tumor cells stimulate adjacent cells to increase MMP-9 production, thereby creating a microenvironment conducive to tumor survival and dissemination, and release growth factors and cytokines that promote carcinogenesis (19,21–23). Its activity is closely linked to angiogenesis, promoting endothelial cell migration, new vessel formation, and the exposure of epitopes that modulate vascular development. At the same time, the enzyme can also generate angiogenesis-inhibiting molecules, such as angiostatin and endostatin, thereby influencing tumor cell apoptosis. MMP-9 also participates in pro- and anti-apoptotic mechanisms, whether through alterations in the extracellular matrix, cleavage of molecules such as Fas, or activation of pathways such as AKT. Specific interactions, such as with endostatin, can modulate its own activation, adjusting cell survival. Thus, MMP-9 functions as a multifunctional regulator of the tumor microenvironment, integrating invasion, metastasis, angiogenesis, and apoptosis, making it an important therapeutic target and prognostic marker across multiple cancer types (23). MMP-9 expression has been described as increased in several tumors, including canine mammary tumors, lymphomas, and melanomas, and is associated with tumor progression and a significant impact on prognosis (24–26).

In human squamous cell carcinoma, MMP-9 expression has been extensively investigated and documented. Several studies show that its expression is increased in tumors when compared to normal tissue, and it is also observed in inflammatory cells (27). However, to our knowledge, no study has evaluated MMP-9 immunohistochemical expression in canine cutaneous squamous cell carcinoma. Thus, the main objective of this study is to analyze MMP-9 expression in this type of neoplasm in dogs.

Material and methods

Biological material

A total of 47 formalin-fixed, paraffin-embedded (FFPE) tissue samples from 47 dogs were analyzed: 10 normal skin samples (without obvious inflammatory or neoplastic pathology) and 37 cutaneous squamous cell carcinomas. All specimens were obtained from clinical cases submitted for routine diagnostic evaluation to the University of Trás-os-Montes and Alto Douro Histology and Pathology Laboratory between 2022 and 2024. Samples consisted of incisional biopsies or surgical excisions from living animals, collected by attending veterinarians, fixed in 10% neutral buffered formalin, and processed and embedded in paraffin wax. For all cases, basic clinical information (including signalment) was available from the laboratory records. Hematoxylin and Eosin (H&E)-stained histological slides were reviewed to confirm the diagnosis and to select representative areas for immunohistochemistry. Twenty cases were in females and seventeen in males. The most common breeds were Boxers (n = 8) and Mixed breed (n = 4), as shown in Table 1. The animals studied ranged in age from 1 to 17 years, with an average age of 8.5 years.

Table 1: Clinical Characteristics, Histological Grade, and MMP-9 Expression Pattern in Dogs with Cutaneous Squamous Cell Carcinoma

Case	Breed	Sex	Age (years)	Histological Grade	MMP-9 (intensity)	MMP-9 (%)
1	Bobtail	Female	10	III	+	diffuse
2	Boxer	Female	8	II	+	diffuse
3	Mixed breed	Female	11	I	+	diffuse
4	Portuguese Pointer	Male	11	II	++	diffuse
5	Bulldog	Male	9	III	++	diffuse
6	Labrador Retriever	Female	7	III	++	diffuse
7	Doberman	Female	6	II	++	diffuse
8	Estrela Mountain Dog	Female	4	III	+++	diffuse
9	Basset Hound	Female	14	II	+++	diffuse
10	Boxer	Female	8	II	+++	diffuse
11	West Highland White Terrier	Male	10	II	+++	diffuse
12	Dalmatian dog	Male	6	I	+	diffuse
13	Castro Laboreiro dog	Male	9	II	+++	diffuse
14	Dalmatian dog	Male	1	III	+++	diffuse
15	Yorkshire	Male	11	III	+++	diffuse
16	Irish Setter	Male	9	II	+++	diffuse
17	Pincher	Female	8	III	+++	diffuse
18	Basset Hound	Male	8	III	+++	diffuse
19	Sharpei	Female	10	III	+++	diffuse
20	Boxer	Male	7	II	+++	diffuse
21	Pit Bull	Male	9	III	+++	diffuse
22	Mixed breed	Female	17	II	+++	diffuse
23	Giant Schnauzer	Male	7	III	+++	diffuse
24	Boxer	Male	10	I	++	diffuse
25	Boxer	Female	9	I	++	diffuse
26	Cocker Spaniel	Female	10	I	+++	diffuse
27	Poodle	Female	12	I	+++	diffuse
28	Boxer	Male	6	II	+++	diffuse
29	German Shepherd	Male	2	II	+++	diffuse
30	Boxer	Female	7	I	+++	diffuse
31	Portuguese Pointer	Female	10	I	+++	diffuse
32	Pekingese	Female	6	II	+++	diffuse
33	Mixed breed	Female	7	I	+++	diffuse
34	Boxer	Male	8	II	+++	diffuse
35	Bichon Frisé	Male	9	I	+++	diffuse
36	Poodle	Female	11	II	+++	diffuse
37	Mixed breed	Female	6	III	+++	diffuse

Histological analysis

Two pathologists (IP and JP) independently analyzed each sample, thoroughly examining all slides in each section. The histopathological diagnosis was based on the World Health Organization (WHO) classification of animal tumors criteria (28), according to the tumor's ontogeny and morphological features. Microscopic observations and image capture were carried out using a Nikon Eclipse E600 microscope with a Nikon DXM1200 digital camera (Nikon Instruments Inc., Melville, NY, USA).

The following histological features were assessed: degree of keratinization, nuclear pleomorphism, and the presence or absence of spindle or anaplastic cells. Keratinization was graded according to the percentage of neoplastic cells exhibiting keratinization into three groups: I (>50% keratinized cells), II (20–50% keratinized cells), and III (0–20% keratinized cells). Nuclear pleomorphism was classified into three categories: I (mild, with >75% of cells showing a mature appearance), II (moderate, with 50–75% mature cells), and III (marked pleomorphism, with <50% mature cells). The overall histological malignancy grade was assigned, with tumors categorized as Grade I, II, or III (Table 2). Grade I (well differentiated) lesions showed nuclear pleomorphism-1, pronounced keratinization, and the absence of spindle or anaplastic cells. Grade II (moderately differentiated) tumors exhibited moderate keratinization, nuclear pleomorphism 1 or 2, and no spindle or anaplastic cells. Grade III (poorly differentiated) lesions displayed rare to absent keratinization, nuclear pleomorphism grade 3, and the presence of spindle or anaplastic cells, and were often difficult to distinguish from SCC.

Table 2: Histological grade of malignancy, adapted from (29).

Histological Grade	Histological Features
Grade I (Well differentiated)	Increased cytoplasm/nucleus ratio (nuclear pleomorphism-1) Pronounced keratinization (1) No spindled or anaplastic cells
Grade II (Moderately differentiated)	Moderate keratinization (2) No spindled or anaplastic cells Nuclear pleomorphism-1 or -2
Grade III (Poorly differentiated)	Rare to absent keratinization (3) Presence of Spindled or anaplastic cells Nuclear pleomorphism-3 Often difficult to distinguish as SCC

Immunohistochemical evaluation of MMP-9

For immunohistochemical analysis, 3-µm-thick sections from paraffin blocks were mounted on silane-coated glass slides. Staining was performed in a humid chamber using a manual immunohistochemistry system (Bio-Optica®, Milan, Italy) with a polymer detection system (Novolink™ Polymer Detection Systems, Leica Biosystems®, Newcastle, UK). The samples were dewaxed and rehydrated in graded alcohol solutions, followed by antigen retrieval in citrate buffer (pH 6.0) with heat treatment in a microwave oven at 750 W for 2 cycles of 5 minutes each. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide (H_2O_2) for 30 minutes, after which the slides were incubated with the Protein Block provided in the Novolink™ Polymer Detection Systems kit (Leica Biosystems®, Newcastle, UK). The samples were then incubated in a humid chamber at 4 °C with the primary anti–MMP-9 antibody (polyclonal, Neomarkers®), which has previously been applied in canine studies (33), at a 1:200 dilution in PBS for approximately 24 hours. This was followed by incubation with the Post Primary reagent for 30 minutes, then with the Novolink™ polymer for an additional 30 minutes, according to the manufacturer's instructions. The reaction was visualized using 3,3'-diaminobenzidine (DAB) as the chromogen (7 minutes), and the slides were counterstained with Gill's hematoxylin for 1 minute, dehydrated, cleared, and mounted with Entellan® resin (Merck®). All immunohistochemical procedures (antigen retrieval, antibody dilution, incubation times, washing steps, and room-temperature conditions) were standardized and performed by the same operator to ensure consistency and reproducibility. Positive controls (canine mammary carcinoma tissue) and negative controls (omission of the primary antibody) were included in each run to ensure the quality and specificity of the immunohistochemical procedure.

Evaluation of MMP-9 immunoreactivity

MMP-9 immunopositivity was identified as brown cytoplasmic staining in neoplastic cells and was evaluated exclusively in tumor cells. The distribution of positive cells was scored based on the percentage of stained tumor cells as follows: negative (0%), focal (1–19%), multifocal (20–49%), and diffuse (≥50%). Staining intensity was graded as absent (0), weak (+), moderate (++), or strong (+++) (30).

All samples were independently evaluated by two blinded observers (IP and JP). Interobserver agreement was assessed using kappa statistics, which indicated substantial to almost perfect agreement across scoring categories. When discrepancies occurred, a third reviewer (CC) re-evaluated the slides, and a consensus was reached to determine the final score.

Statistical Analysis

All statistical analyses were conducted using SPSS software (version 21; SPSS Inc., Chicago, Illinois, USA). The chi-square (χ^2) test was applied to examine the relationship between MMP-9 and the histological grade of malignancy. A p-value below 0.05 was considered indicative of statistical significance.

Results

Histopathological Evaluation

Among the analyzed tumors, 10 cases (27.03%) were classified as well-differentiated grade I, 15 cases (40.54%) as moderately differentiated grade II, and 12 cases (32.43%) as poorly differentiated grade III, as shown in Figure 1.

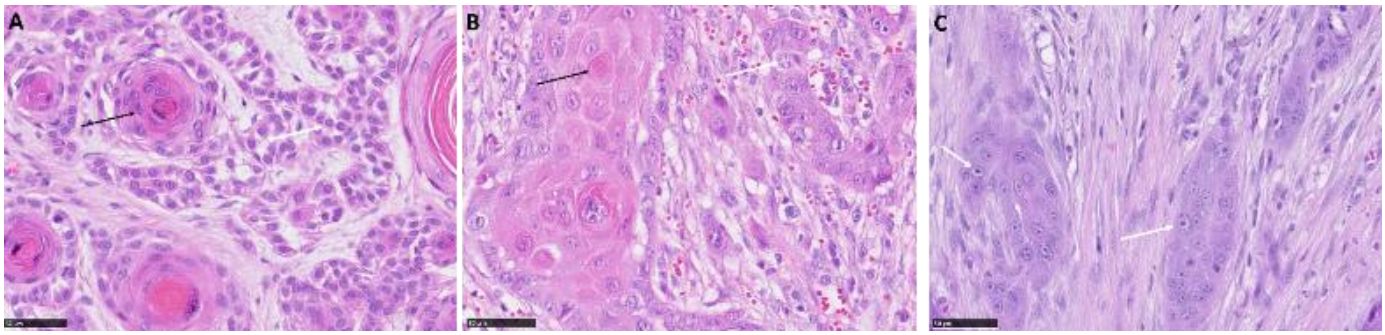

Figure 1: Histopathological features of canine cutaneous squamous cell carcinoma (H&E). (A) Well-differentiated (grade I), showing prominent keratinization with keratin pearl formation (black arrow) and mild nuclear pleomorphism (white arrow), 400x. (B) Moderately differentiated (grade II), characterized by irregular nests of squamous cells with moderate keratinization (black arrow) and moderate nuclear pleomorphism (white arrow), 400x. (C) Poorly differentiated (grade III), displaying poorly defined sheets of malignant cells with rare to absent keratinization and marked nuclear pleomorphism (white arrows), 400x.

MMP-9 immunohistochemical evaluation

In the 10 cases of normal skin analyzed, no expression of this molecule was detected. Regarding the tumors, all exhibited MMP-9 immunoexpression in both tumor cells and the stroma, including inflammatory cells such as macrophages (Figure 2). In tumoral cells, staining was diffuse and present in more than half of the cells, regardless of histological grade.

For staining intensity, 27 tumors showed strong intensity (+++), 6 moderate intensity (++), and 4 weak intensity (+). In Grade I tumors, 20% (2/10) showed weak (+) staining, 20% (2/10) had moderate (++) staining, and 60% (6/10) exhibited strong (+++) staining.

In Grade II tumors, 6.7% (1/15) showed weak (+) staining, 13.3% (2/15) moderate (++) staining, and 80.0% (12/15) strong (+++) staining.

In Grade III tumors, 8.3% (1/12) showed weak (+) staining, 25% (2/12) had moderate (++) staining, and 66.7% (9/12) exhibited strong (+++) staining. Across all histological grades, strong staining intensity predominated, and no significant association was observed between MMP-9 immunohistochemical staining and histopathological grade (p=0.81).

Discussion

Given the local aggressiveness of cutaneous squamous cell carcinoma, it is essential to understand the mechanisms of invasion and tumor progression and to investigate molecules that could serve as therapeutic targets (31). In this context, MMP-9, a matrix metalloproteinase, plays a crucial role in degrading the extracellular matrix. Its expression is associated with important tumor events. MMP-9, a gelatinase, participates not only in cancer invasion and metastasis by degrading type IV collagen in the basement membrane and the extracellular matrix, but also in angiogenesis and the growth of cancer cells (24,32–34).

In this study, normal skin samples were negative for MMP-9 expression, consistent with human studies showing that MMP-9 is typically absent in non-tumoral epithelium (31,35).

Among the 37 tumors analyzed, all were positive for MMP-9, and 27 showed high expression. Its presence was also detected in the tumor stroma. Although no previous studies on canine cutaneous SCC allow direct comparison with our results, research on other canine tumors has reported observations similar to ours. For example, cartilaginous bone tumors, including chondroblastic osteosarcomas, chondrosarcomas (36), and multilobular bone tumors (36), showed higher MMP-9 expression compared to benign tumors and non-tumoral tissues. Similarly, canine and feline osteosarcomas (37) exhibited positivity for MMP-9. Similar results were observed in mammary tumors (37), where MMP-9 expression was higher in malignant tumors than in benign ones and was associated with the presence of this protein in the tumor stroma. Additionally, in canine mast cell tumors and melanocytic tumors, the intensity of MMP-9 expression was higher in tumors with more aggressive behavior (38).

Regarding human squamous cell carcinoma, the findings on MMP-9 expression are not fully consistent across studies. Similar results were reported in cutaneous SCC (39,40), esophageal SCC (41), oral SCC (42,43), oral SCC of the tongue (44), and squamous cell carcinoma of the head and neck (45,46). Conversely, a study of oral SCC reported a higher proportion of cases with weak-to-absent MMP-9 expression (47). Similarly, in head and neck carcinoma, more cases were negative for MMP-9 than positive (48). This discrepancy between human and animal studies and our findings may reflect differences in sample size, individual variability, anatomic location, or species-specific aspects of tumor biology. Methodological factors may also contribute, including variations in immunohistochemical protocols (such as biotin–streptavidin versus polymer-based systems). Additionally, the antibody used is not canine-specific, as is the case for most human-developed antibodies. We were also unable to control the fixation time prior to sample arrival at the laboratory, which may have influenced the staining results.

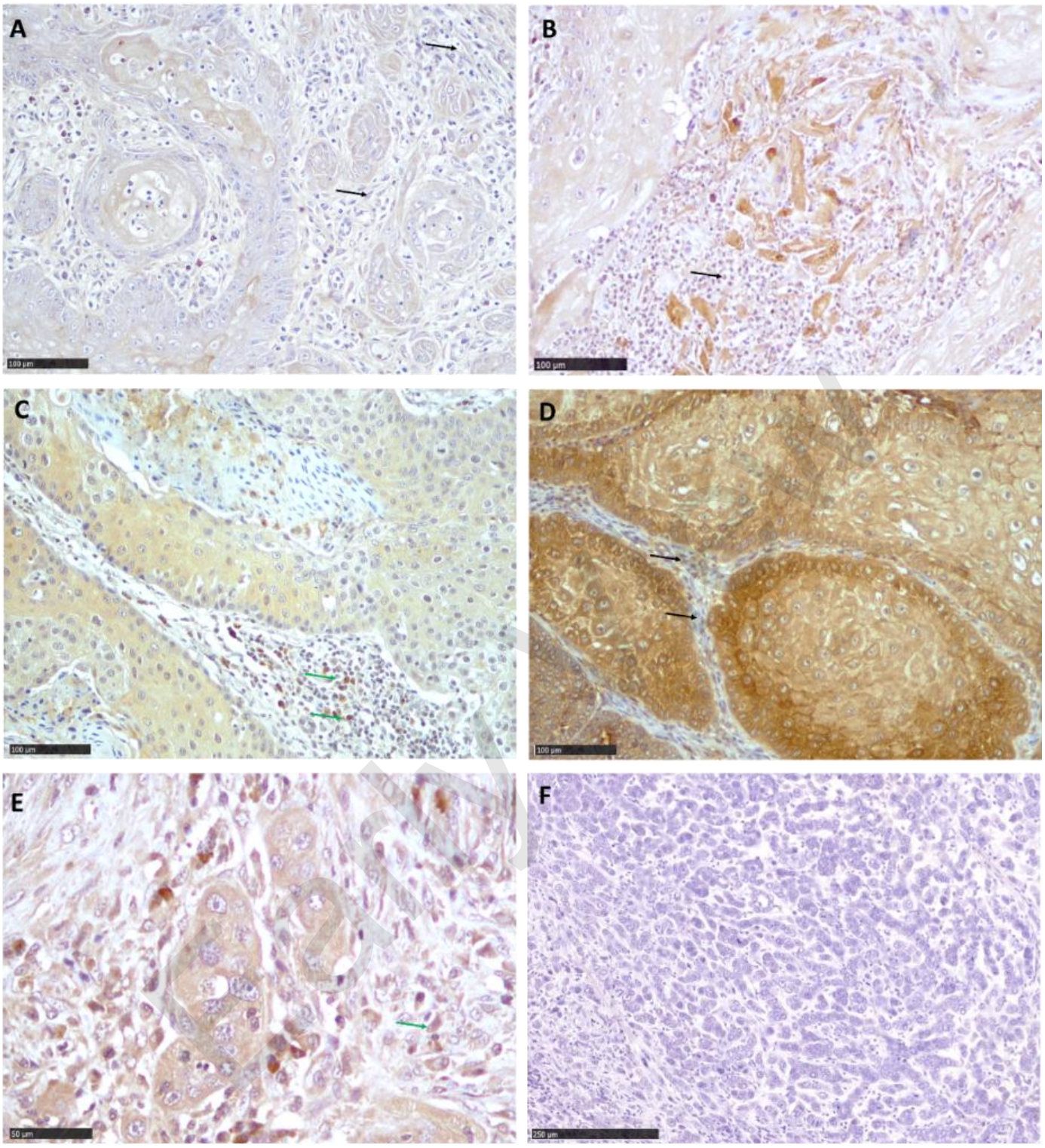

Figure 2: Immunoexpression of MMP-9 in canine cutaneous squamous cell carcinoma (SCC). Positivity is observed in tumor cells, the stroma (black arrow), and macrophages (green arrow). (A) Weak staining intensity in well-differentiated SCC (grade I); 200x. (B) Weak staining intensity in well-differentiated SCC (grade I), stromal positivity is also present (black arrow), 200x. (C) Moderate staining intensity in moderately differentiated SCC; 200x. (D). Strong staining in tumor islands of moderately differentiated SCC; 200x. (E) Strong staining intensity in poorly differentiated SCC, 400x. (F) Negative control showing absence of MMP-9 expression, 100x.

Regarding a potential association with tumor aggressiveness, in our study, no differences were observed among histological grades of malignancy, with MMP-9 staining intensity (+++) observed across all differentiation grades. In humans, the prognostic value of MMP-9 remains controversial, with some studies reporting correlations with aggressive tumor behavior (47,49), whereas others, similar to this study, do not find a significant association (45,46). Despite the lack of a significant correlation with histological grade, the diffuse and strong expression of MMP-9 in canine cutaneous SCC suggests it may be a potential therapeutic target. MMP inhibitors, which have shown promise in limiting tumor invasion and metastasis in human studies, may also hold potential in veterinary oncology (18,50–52).

Additionally, a clear difference between normal and neoplastic epidermis is observed, with MMP-9 being expressed only in tumor tissues. This reinforces the hypothesis that MMP-9 expression is closely linked to pathological processes, particularly those involved in oncogenesis and tumor progression. In both canine and human SCC, MMP-9 may play a critical role in degrading the extracellular matrix, thereby facilitating tumor cell invasion (53,54). However, as it is also expressed in low-grade tumors, MMP-9 expression may represent an early event in neoplastic transformation, as suggested by human studies, highlighting its potential role in early tumor progression (21,40,55).

Although it was not quantified, MMP-9 labeling was also observed in the stroma and macrophages, suggesting that MMP-9 is produced by both neoplastic cells and the stroma. This finding highlights the tumor as a dynamic ecosystem in which tumor and non-tumor cells influence biological behavior. According to other studies, its presence in inflammatory cells supports the notion that MMP-9 arises from interactions between tumor and non-tumor cells and plays an important role in tumor progression (50).

Several biological mechanisms may be implicated in the association between MMP-9 expression and tumor aggressiveness, an interesting area of investigation in canine squamous cell carcinomas. These mechanisms include the enzyme's capacity to remodel the extracellular matrix, promote angiogenesis, and regulate immune cell dynamics within the tumor microenvironment (51,56). The relationship between increased MMP-9 secretion and tissue damage during cancer is well documented. By degrading extracellular matrix components, MMP-9 enables the reorganization of structural proteins and the removal of physical barriers that restrict cell migration. Disruption of the basement membrane, composed of laminins and cross-linked collagens, facilitates the spread of invasive cancer cells, the recruitment of immune cells to the tumor stroma, and the formation of new blood and lymphatic vessels, processes that are essential for supplying the tumor with nutrients and oxygen (50,51,57).

In addition, MMP-9 can cleave extracellular signaling molecules, such as CXC chemokines, thereby regulating immune cell trafficking and acting as an important coordinator of the tumor microenvironment (57,58). Increased MMP-9 expression has also been positively correlated with the infiltration of B cells, $CD8^+$ and $CD4^+$ T lymphocytes, and macrophages in cancer types such as adrenocortical carcinoma and renal clear cell carcinoma (51,53,57,58).

Our study has several limitations, including the relatively small number of tumors analyzed and the absence of follow-up cases. In addition, although MMP-9 expression was observed in stromal and inflammatory cells, a systematic evaluation of these compartments was not performed. A more detailed characterization of these cell populations, along with exploration of associated mechanisms such as angiogenesis, could further enhance understanding of MMP-9 activity in cutaneous SCC. Moreover, immunohistochemical studies in veterinary medicine often lack control over important pre-analytical variables, such as fixation time, and are limited by the reduced availability of canine-specific antibodies, which may affect staining standardization and interpretation.

In this study, we conducted a preliminary analysis of MMP-9 immunohistochemical expression, suggesting increased expression in tumor cells, stromal cells, and macrophages. These findings lay the groundwork for future research to gain deeper insights into the role of MMP-9 in tumor progression and the microenvironment of squamous cell carcinoma.

Conclusion

MMP-9 is associated with the progression and metastasis of malignant tumors in humans and dogs, but its expression in canine tumors, such as cutaneous squamous cell carcinoma (SCC), is still poorly investigated. This study revealed strong MMP-9 expression in canine SCC, highlighting its role in the tumor microenvironment. However, in our study, MMP-9 expression was not associated with the histological grade of malignancy, underscoring the complexity of its involvement in tumor behavior. Future research should examine the relationship between MMP-9 expression and tumor progression, as well as the underlying mechanisms, including immune infiltration and angiogenesis.

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Ethical statement. Not applied. The authors declare that they have no conflict of interest..

References

1. Webster JD, Solon M, Gibson-Corley KN. Validating Immunohistochemistry Assay Specificity in Investigative Studies: Considerations for a Weight of Evidence Approach. Vet Pathol 2021; 58(5): 829–40. doi:10.1177/0300985820960132.

2. Marques GR, Rocha LF, Vargas THM, Pulz LH, Huete GC, Cadrobbi KG, et al. Relationship of Galectin-3 Expression in Canine Cutaneous Squamous Cell Carcinomas with Histopathological Grading and Proliferation Indices. J Comp Pathol 2020;178:16–21. doi: 10.1016/j.jcpa.2020.06.004.

3. Chikweto A, McNeil P, Bhaiyat MI, Stone D, Sharma RN. Neoplastic and Non-neoplastic Cutaneous Tumors of Dogs in Grenada, West Indies. ISRN Vet Sci 2011;2011:1–6. doi:10.5402/2011/416435.

4. Cardoso C, Files R, Silva F, Barbedo P, Prada J, Pires I. Knowledge, Perceptions, Attitudes, and Practices of Dog and Cat Owners Regarding Skin Tumors: A Cross-Sectional Study. Vet Sci 2025; 12(11):1020. doi: 10.3390/vetsci12111020

5. De Almeida EMP, Piché C, Sirois J, Doré M. Expression of Cyclo-oxygenase-2 in Naturally Occurring Squamous Cell Carcinomas in Dogs. J Histochem Cytochem 2001; 49(7): 867–75. doi: 10.1177/002215540104900707

6. Alves CDBT, Weber MN, Guimarães LLB, Cibulski SP, Da Silva FRC, Daudt C, et al. Canine papillomavirus type 16 associated to squamous cell carcinoma in a dog: virological and pathological findings. Braz J Microbiol 2020; 51(4): 2087–94. doi: 10.1007/s42770-020-00310-4

7. Bongiovanni L, Colombi I, Fortunato C, Salda LD. Survivin expression in canine epidermis and in canine and human cutaneous squamous cell carcinomas. Vet Dermatol 2009; 20(5–6): 369–76. doi: 10.1111/j.1365-3164.2009.00822.x

8. Ortloff A, Bustamante FA, Molina L, Ojeda J, Figueroa CD, Ehrenfeld P. Kallikrein-related Peptidase 5 (KLK5) Expression and Distribution in Canine Cutaneous Squamous Cell Carcinoma. J Comp Pathol 2020; 174: 113–9.doi: 10.1016/j.jcpa.2019.11.009

9. Liffman R, Courtman N. Fine needle aspiration of abdominal organs: a review of current recommendations for achieving a diagnostic sample. J Small Anim Pract 2017; 58(11): 599–609. doi: 10.1111/jsap.12709

10. Bahr KL, Sharkey LC, Murakami T, Feeney DA. Accuracy of US-Guided FNA of Focal Liver Lesions in Dogs: 140 Cases (2005–2008). J Am Anim Hosp Assoc 2013; 49(3): 190–6. doi: 10.5326/JAAHA-MS-5851

11. Diamantis A, Magiorkinis E, Koutselini H. Fine-needle aspiration (FNA) biopsy: historical aspects. Folia Histochem Cytobiol 2009; 47(2): 191–7.doi: 10.2478/v10042-009-0027-x

12. Siddig E. Fine needle aspiration: past, current practice and recent developments. Biotech Histochem 2014; 89(4): 241–4.

13. Dufour JP, Allers C, Schiro F, Falkenstein KP, Gregoire KK, Glover CD, et al. Comparison of fine-needle aspiration techniques. J Med Primatol 2023; 52(6): 400–4.

14. Vandana G, Magar SLR, Anil S, Rani S. Evaluation of histopathological findings of skin biopsies in various skin disorders. Perspect Med Res 2017; 5(2): 37–40.

15. Ito T, Tsuji G, Ohno F, Nakahara T, Uchi H, Furue M. Potential role of the OVOL1–OVOL2 axis and c-Myc in the progression of cutaneous squamous cell carcinoma. Mod Pathol 2017; 30(7): 919–27.

16. Jorgenson E, Choquet H, Yin J, Asgari MM. Common Mitochondrial Haplogroups and Cutaneous Squamous Cell Carcinoma Risk. Cancer Epidemiol Biomarkers Prev 2018; 27(7): 838–41.

17. Zhang H, Liu L, Liu J, Dang P, Hu S, Yuan W, et al. Roles of tumor-associated macrophages in anti-PD-1/PD-L1 immunotherapy for solid cancers. Mol Cancer 2023; 22(1): 58.

18. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors 2018; 18(10): 3249. doi:10.3390/s18103249.

19. Ram M, Sherer Y, Shoenfeld Y. Matrix Metalloproteinase-9 and Autoimmune Diseases. J Clin Immunol 2006; 26(4): 299–307. doi:10.1007/s10875-006-9022-6.

20. Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of Matrix Metalloproteinases in Photoaging and Photocarcinogenesis. IJMS 2016; 17(6): 868. doi:10.3390/ijms17060868

21. Mondal S, Adhikari N, Banerjee S, Amin SA, Jha T. Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview. Eur J Med Chem 2020; 194: 112260. doi: 10.1016/j.ejmech.2020.112260.

22. Kumar NR, Praveen M, Narasimhan R, Khamar P, D'Souza S, Sinha-Roy A, et al. Tear biomarkers in dry eye disease: Progress in the last decade. Indian J Ophthalmol 2023; 71(4): 1190–202. doi: 10.4103/IJO.IJO_2981_22

23. Takai S, Jin D. Pathophysiological Role of Chymase-Activated Matrix Metalloproteinase-9. Biomedicines 2022; 10(10): 2499.doi: 10.3390/biomedicines10102499

24. Raposo TP, Beirão BC, Pires I, Prada J, Brilhante P, Argyle DJ, Queiroga FL. Immunohistochemical expression of CCR2, CSF1R and MMP9 in canine inflammatory mammary carcinomas. Anticancer Res 2016; 36(4): 1805–13.

25. Docampo MJ, Cabrera J, Rabanal RM, Bassols A. Expression of matrix metalloproteinase-2 and -9 and membrane-type 1 matrix metalloproteinase in melanocytic tumors of dogs and canine melanoma cell lines. AJVR 2011; 72(8): 1087–96. doi:10.2460/ajvr.72.8.1087.

26. Aresu L, Aricò A, Comazzi S, Gelain ME, Riondato F, Mortarino M, et al. VEGF and MMP -9: biomarkers for canine lymphoma. Vet Comp Oncol 2014; 12(1): 29–36.doi: 10.1111/j.1476-5829.2012.00328.x

27. Ahmed Haji Omar A, Haglund C, Virolainen S, Häyry V, Atula T, Kontio R, et al. MMP-7, MMP-8, and MMP-9 in oral and cutaneous squamous cell carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol 2015; 119(4): 459–67. doi: 10.1016/j.oooo.2014.12.019

28. Goldschmidt MH, Kiupel M, Foundation DT, Klopfleisch R, Munday JS, Sruggs JL. Surgical Pathology of Tumors of Domestic Animals Volume 1: Epithelial Tumors of the Skin. Davis Thompson Foundation; 2018.

29. Diede C, Walker T, Carr DR, Shahwan KT. Grading differentiation in cutaneous squamous cell carcinoma: a review of the literature. Arch Dermatol Res 2024; 316(7): 434. doi:10.1007/s00403-024-03184-w.

30. Kosunen A, Pirinen R, Ropponen K, Pukkila M, Kellokoski J, Virtaniemi J, et al. CD44 expression and its relationship with MMP-9, clinicopathological factors and survival in oral squamous cell carcinoma. Oral Oncol 2007; 43(1): 51–9. doi: 10.1016/j.oraloncology.2006.01.003.

31. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. J Pathol 2003; 200 (4): 448–64. doi:10.1002/path.140

32. Gong L, Wu D, Zou J, Chen J, Chen L, Chen Y, et al. Prognostic impact of serum and tissue MMP-9 in non-small cell lung cancer: a systematic review and meta-analysis. Oncotarget 2016; 7(14): 18458–68. doi:10.18632/oncotarget.7607.

33. Goździalska A, Wojas-Pelc A, Drąg J, Brzewski P, Jaśkiewicz J, Pastuszczak M. Expression of metalloproteinases (MMP-2 and MMP-9) in basal-cell carcinoma. Mol Biol Rep 2016; 43(10): 1027–33. doi:10.1007/s11033-016-4040-9.

34. Hadler-Olsen E, Winberg JO, Uhlin-Hansen L. Matrix metalloproteinases in cancer: their value as diagnostic and prognostic markers and therapeutic targets. Tumor Biol 2013; 34(4): 2041–51. doi:10.1007/s13277-013-0842-8.

35. Sternlicht MD, Werb Z. How Matrix Metalloproteinases Regulate Cell Behavior. Annu Rev Cell Dev Biol 2001; 17(1): 463–516. doi: 10.1146/annurev.cellbio.17.1.463.

36. Loukopoulos P, Mungall BA, Straw RC, Thornton JR, Robinson WF. Matrix Metalloproteinase-2 and −9 Involvement in Canine Tumors. Vet Pathol 2003; 40(4): 382–94. doi: 10.1354/vp.40-4-382

37. Gebhard C, Fuchs-Baumgartinger A, Razzazi-Fazeli E, Miller I, Walter I. Distribution and activity levels of matrix metalloproteinase 2 and 9 in canine and feline osteosarcoma. Can J Vet Res 2016; 80(1): 66–73.

38. Leibman NF, Lana SE, Hansen RA, Powers BE, Fettman MJ, Withrow SJ, et al. Identification of Matrix Metalloproteinases in Canine Cutaneous Mast Cell Tumors. J Vet Intern Med 2000;14(6):583–6. doi: 10.1892/0891-6640(2000)014<0583:iommic>2.3.co;2

39. Hernández-Pérez M, El-hajahmad M, Massaro J, Mahalingam M. Expression of Gelatinases (MMP-2, MMP-9) and Gelatinase Activator (MMP-14) in Actinic Keratosis and in In Situ and Invasive Squamous Cell Carcinoma. Am J Dermatopathol 2012; 34(7): 723–8. doi: 10.1097/DAD.0b013e31824b1ddf.

40. Poswar FO, Fraga CAC, Farias LC, Feltenberger JD, Cruz VPD, Santos SHS, et al. Immunohistochemical analysis of TIMP-3 and MMP-9 in actinic keratosis, squamous cell carcinoma of the skin, and basal cell carcinoma. Pathol Res Pract 2013; 209(11): 705–9. doi: 10.1016/j.prp.2013.08.002.

41. Li Y, Ma J, Guo Q, Duan F, Tang F, Zheng P, et al. Overexpression of MMP-2 and MMP-9 in esophageal squamous cell carcinoma. Dis Esophagus 2009; 22(8): 664–7. doi:10.1111/j.1442-2050.2008.00928. x.

42. Maurya SB, Shetty DC, Rathore AS, Juneja S, Jain A, Banga A. Immunolocalization of epithelial cell adhesion molecule and matrix metalloproteinase-9 in oral epithelial dysplasia and oral squamous cell carcinoma. J Cancer Res Ther 2023; 19(7): 1775–80. doi: 10.4103/jcrt.jcrt_1012_21.

43. Monteiro L, Delgado M, Ricardo S, Do Amaral B, Salazar F, Pacheco J, et al. Prognostic significance of CD44v6, p63, podoplanin and MMP-9 in oral squamous cell carcinomas. Oral Diseases 2016; 22(4): 303–12. doi:10.1111/odi.12442.

44. Fan HX, Li HX, Chen D, Gao ZX, Zheng JH. Changes in the expression of MMP2, MMP9, and ColIV in stromal cells in oral squamous tongue cell carcinoma: relationships and prognostic implications. J Exp Clin Cancer Res 2012; 31(1): 90. doi:10.1186/1756-9966-31-90.

45. Ondruschka C, Buhtz P, Motsch C, Freigang B, Schneider-Stock R, Roessner A, et al. Prognostic Value of MMP-2, -9 and TIMP-1,-2 Immunoreactive Protein at the Invasive Front in Advanced Head and Neck Squamous Cell Carcinomas. Pathol Res Pract 2002; 198(8): 509–15. doi:10.1078/S0344-0338(04)70292-7.

46. Ruokolainen H, Paakko P, Turpeenniemi-Hujanen T. Expression of Matrix Metalloproteinase-9 in Head and Neck Squamous Cell Carcinoma: A Potential Marker for Prognosis. Clin Cancer Res 2004; 10(9): 3110–6.

47. Akdeniz O, Akduman D, Haksever M, Ozkarakas H, Muezzinoglu B. Relationships Between Clinical Behavior of Laryngeal Squamous Cell Carcinomas and Expression of VEGF, MMP-9 and E-Cadherin. Asian Pac J Cancer Prev 2013; 14(9): 5301–10. doi:10.7314/APJCP.2013.14.9.530

48. You TK, Kim KM, Noh SJ, Bae JS, Jang KY, Chung MJ, et al. Expressions of E-cadherin, Cortactin and MMP-9 in Pseudoepitheliomatous Hyperplasia and Squamous Cell Carcinoma of the Head and Neck: Their Relationships with Clinicopathologic Factors and Prognostic Implication. Korean J Pathol 2012; 46(4): 331. doi:10.4132/KoreanJPathol.2012.46.4.331.

49. Mohamed MF, Al-Khudari S, Cassini-Vieira P, Erra A, Bagabas R, Houser T, et al. Presence of CrkI-containing microvesicles in squamous cell carcinomas could have ramifications on tumor biology and cancer therapeutics. Sci Rep 2022; 12(1): 4803. doi:10.1038/s41598-022-08905-7.

50. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2(3): 161–74. doi:10.1038/nrc745.

51. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J 2011; 278(1): 16–27. doi:10.1111/j.1742-4658.2010.07919. x.

52. Giantin M, Aresu L, Benali S, Aricò A, Morello EM, Martano M, et al. Expression of Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases and Vascular Endothelial Growth Factor in Canine Mast Cell Tumours. J Comp Pathol 2012; 147(4): 419–29. doi: 10.1016/j.jcpa.2012.01.011.

53. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. Amino Acids 2011;41(2):271–90. doi:10.1007/s00726-010-0689-x.

54. Verdolini R, Amerio P, Goteri G, Bugatti L, Lucarini G, Mannello B, et al. Cutaneous carcinomas and preinvasive neoplastic lesions. Role of MMP-2 and MMP-9 metalloproteinases in neoplastic invasion and their relationship with proliferative activity and p53 expression. J Cutan Pathol 2001; 28(3): 120–6. doi:10.1034/j.1600-0560.2001.028003120. x.

55. Ries C, Popp T, Egea V, Kehe K, Jochum M. Matrix metalloproteinase-9 expression and release from skin fibroblasts interacting with keratinocytes: Upregulation in response to sulphur mustard. Toxicology 2009; 263(1): 26–31. doi: 10.1016/j.tox.2008.08.011.

56. Henriques ÁCG, De Matos FR, Galvão HC, Freitas RDA. Immunohistochemical expression of MMP-9 and VEGF in squamous cell carcinoma of the tongue. J Oral Sci 2012; 54(1): 105–11. doi:10.2334/josnusd.54.105.

57. Augoff K, Hryniewicz-Jankowska A, Tabola R, Stach K. MMP9: A Tough Target for Targeted Therapy for Cancer. Cancers 2022; 14(7): 1847. doi:10.3390/cancers14071847

58. Zeng Y, Gao M, Lin D, Du G, Cai Y. Prognostic and Immunological Roles of MMP-9 in Pan-Cancer. Hu WM, editor. Biomed Res Int 2022; 2022(1): 2592962. doi:10.1155/2022/2592962.