

OPEN ACCESS

EDITED BY

Francisco José Pallarés,
University of Cordoba, Spain

REVIEWED BY

Fernando Leiva-Cepas,
Reina Sofia University Hospital, Spain
Jacinto Da Costa Silva Neto,
Federal University of Pernambuco, Brazil

*CORRESPONDENCE

Florentina Daraban Bocaneti
✉ florentinabocaneti@yahoo.com

*PRESENT ADDRESS

Gennaro Altamura,
Department of Agricultural, Food, and
Forest Sciences, University of Palermo,
Palermo, Italy

[†]These authors have contributed equally
to this work and share first authorship

RECEIVED 14 January 2026

REVISED 19 March 2026

ACCEPTED 26 March 2026

PUBLISHED 10 April 2026

CITATION

Tutu P, Altamura G, Daraban Bocaneti F,
Hritcu OM, Pasca A-S, Dascalu MA,
Horodincu L, Tanase OI, Mares M and
Borzacchiello G (2026)
Immunohistochemical and western blot
expression of MMPs, TIMPs, and
cytokeratin 10 in feline squamous cell
carcinoma.
Front. Vet. Sci. 13:1787600.
doi: 10.3389/fvets.2026.1787600

COPYRIGHT

© 2026 Tutu, Altamura,
Daraban Bocaneti, Hritcu, Pasca,
Dascalu, Horodincu, Tanase, Mares and
Borzacchiello. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication
in this journal is cited, in accordance
with accepted academic practice. No
use, distribution or reproduction is
permitted which does not comply with
these terms.

Immunohistochemical and western blot expression of MMPs, TIMPs, and cytokeratin 10 in feline squamous cell carcinoma

Paul Tutu^{1†}, Gennaro Altamura^{2†*}, Florentina Daraban Bocaneti^{1*},
Ozana Maria Hritcu¹, Aurelian-Sorin Pasca¹,
Mihaela Anca Dascalu¹, Loredana Horodincu¹,
Oana Irina Tanase¹, Mihai Mares¹ and Giuseppe Borzacchiello²

¹“Ion Ionescu de la Brad” Iasi University of Life Sciences, Iasi, Romania, ²Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Napoli, Italy

Background: Feline oral and cutaneous squamous cell carcinoma (SCC) are aggressive neoplasms characterized by high local invasiveness. Given its spontaneous occurrence and molecular similarities, feline SCC represents a robust comparative model for human head and neck squamous cell carcinoma (HNSCC). This pilot study aimed to characterize the expression of metalloproteinases (MMPs), their tissue inhibitors (TIMPs), and cytokeratin 10 (CK10) in relation to Papillomavirus (PV) status, and to explore the viral-molecular relationship in feline oncology.

Methods: Ten feline SCC samples (3 oral and 7 cutaneous) were analyzed. PV DNA was detected using PCR with degenerated primers FAP59/FAP64. Protein expression of MMP-2, -9, -13, -14, TIMP-2, -3, and CK10 was analyzed and quantified via immunohistochemistry (IHC) (*H*-score) and validated through Western blot analysis. Statistical correlations were determined using the Spearman rank correlation coefficient, and statistical differences between groups were assessed via Mann–Whitney *U* test.

Results: PV DNA was identified in 6/10 samples. IHC analysis revealed that MMPs (-2, -9, -13, -14) exhibited stronger cytoplasmic and nuclear immunostaining compared to the more restricted signals of TIMPs and CK10, showing a strong inverse correlation with MMP-2, linking invasiveness to dedifferentiation. Significant positive correlations were found between MMP-9/TIMP-3 and MMP-2/MMP-9. TIMP-2 expression was significantly higher in PV-negative samples. Western blot confirmed these results, showing consistent bands.

Conclusion: This pilot study provides the first characterization of MMPs, TIMPs, and CK10 in feline SCC, establishing a foundation for future research into feline PV species and reinforce the value of the feline model in comparative oncology.

KEYWORDS

squamous cell carcinoma, cytokeratin, feline, matrix metalloproteinases, papillomavirus

1 Introduction

Feline cutaneous and oral squamous cell carcinoma (CSCC, OSCC) are common and aggressive tumors in cats, often linked to PV infection (1–3). SCC accounts for about 15% of cutaneous and 60%–75% of oral tumors in felines (4, 5). Beyond its veterinary importance, feline OSCC represents a robust model for HHNSCC due to similarities in clinical behavior, molecular profile, and risk factors (6, 7). This comparative pathology approach suggests that investigating molecular mechanisms in the feline model may directly benefit oncological progress in both veterinary and human medicine (8).

MMPs, particularly MMP-2 and MMP-9, and their inhibitors regulate tumor invasion and progression, with imbalances linked to cancer aggressiveness (9, 10). Similar mechanisms have been observed in bovine fibropapillomas associated with papillomavirus infection (11, 12).

CK10 marks epithelial differentiation, while its loss is correlated with tumor progression and aggressiveness in SCC (13, 14). An inverse relationship between MMP-9 and CK10 expression in tumorigenic keratinocytes suggests that decreased differentiation is linked to higher invasive potential (15). Although MMPs are known markers of invasion (16), this specific panel (MMP-2, 9, 13, 14, TIMP-2, 3, and CK10) has never been investigated in relation to PV status in cats.

Therefore, this pilot study aimed at evaluating the immunohistochemical and Western Blot expression of MMP-2, -9, -13, -14, TIMP-2, -3, and CK10 in a cohort of feline SCCs (cutaneous and oral), correlating these molecular profiles with PV status (detected by PCR). Our objective was to identify co-expression patterns that could serve as indicators of virus-influenced tumor progression.

2 Methods

2.1 Samples

A total of 10 SCC samples collected from cats were analyzed. Five cases were retrieved from the Infectious Diseases Clinic, Faculty of Veterinary Medicine, Iasi, Romania, where each sample was divided into two parts: one part stored at -80°C for biochemical assays and the other fixed in 10% buffered formalin for histopathological analysis. The remaining five cases were retrieved from the archives of the Anatomical Pathology Department of the same institution, and included only as paraffin-embedded samples. For molecular biology studies, 20 μm -thick sections were cut from each paraffin block using separate microtome blades and collected in sterile tubes to prevent cross-contamination.

2.2 PCR

Genomic DNA was isolated from the SCC samples using the Qiagen DNA Mini Kit following the manufacturer's instructions. PV DNA detection was performed by conventional PCR targeting a conserved ~478 bp fragment of the L1 gene. The analysis utilized the degenerate primers FAP59 (5'-TAACWGTIGGICAYCCWTATT-3') and FAP64 (5'-CCWATATCWVHCATITCICCATC-3'), as described by (17).

The PCR amplification was carried out in a final volume of 20 μL containing: 5 μL of extracted DNA, 1X PCR buffer (Invitrogen, Platinum II Hot-Start Green PCR Master Mix) and 0.75 μM of each primer. The thermal cycling conditions consisted of an initial denaturation at 94°C for 10 min, followed by 45 cycles of denaturation at 94°C for 90 s, annealing at 50°C for 90 s, and extension at 72°C for 90 s, with a final extension step at 72°C for 5 min.

To ensure the reliability of the results, a negative control (nuclease-free water) was included in each run. The amplified products were visualized by electrophoresis on a 2% agarose gel stained with SYBR Safe DNA Gel Stain (Invitrogen) and compared against a 100 bp DNA ladder (Invitrogen). Digital representation of the gel was captured and analyzed using the iBright FL1500 Imaging System (Invitrogen).

2.3 Histopathology

The samples were fixed in 10% buffered formalin and then embedded in paraffin for routine histological processing and staining. Light microscopy evaluation was performed according to the guideline proposed by (18) using hematoxylin and eosin staining.

2.4 Immunohistochemistry

Paraffin-embedded sections from 10 SCC samples were subjected to immunohistochemistry using the streptavidin-biotin-peroxidase method (Novolink Polymer Detection System; Leica Biosystems, Newcastle, United Kingdom), following the protocol described by (11). Primary antibodies against MMP-2 (PA5-16504, Thermo Fisher), MMP-9 (PA5-27191, Thermo Fisher), MMP-13 (MA5-14238, Thermo Fisher), and MMP-14 (PA5-104459, Thermo Fisher) were applied for 1 h at room temperature. Anti-TIMP-2 (MA5-12207, Thermo Fisher) and anti-TIMP-3 (PA5-70423, Thermo Fisher) antibodies were incubated overnight at 4°C , as described by (11, 12, 19). Anti-CK10 (MA5-42858, Thermo Fisher) was applied overnight at 4°C at 1:200 dilution. Diaminobenzidine was used to visualize specific immunoreactivity. The protocol included bovine cutaneous fibropapilloma samples as positive controls, which have been shown to express these MMPs and TIMPs (11, 12), while for the negative controls the primary antibodies were omitted and substituted with phosphate-buffered saline. Immunoreactivity was analyzed using QuPath 0.5.1 open-source software, with digital image analysis performed on 3 different images at 40 $\times$ magnification. The *H*-score was calculated using the method described by (20): $(1 \times \text{percentage of weak staining}) + (2 \times \text{percentage of moderate staining}) + (3 \times \text{percentage of strong staining})$ within the target region, yielding scores from 0 to 300.

2.5 Western blotting

Biochemical analysis was conducted on 5 tissue samples of feline SCC. Protein extraction, electrophoresis, blotting, and background blocking were carried out following the protocol described by (21). The membranes were incubated with the following primary antibodies: anti-MMP-2 (1:500), anti-MMP-9 (1:1000), anti-MMP-13 (1:1000), anti-MMP-14 (1:1000), anti-TIMP-2 (1:500), anti-TIMP-3 (1:500), and anti-Cytokeratin10 (1:500). After washing with TBS-0.1% Tween buffer, HRP-conjugated secondary antibodies, horse anti-mouse (Cell Signaling Technology #7076S) and goat anti-rabbit (Cell Signaling Technology #7074S), were applied for 1 h at room

temperature. Protein bands were detected via enhanced chemiluminescence (ECL, Bio-Rad) using a ChemiDoc gel scanner (Bio-Rad). To ensure comparable protein loading and for normalization of densitometric data, β -actin was used as the internal reference protein (housekeeping protein), membranes were stripped and incubated with mouse anti- β -actin antibody (C-2: sc-8432, Santa Cruz) at a 1:500 dilution. Densitometric quantification of proteins was performed using Image Lab software (Bio-Rad), with protein levels normalized to β -actin and expressed as densitometric ratios.

2.6 Statistical analysis

Statistical analysis was performed using SPSS version 26.0. Given the small sample size ($n = 10$) and the exploratory nature of this pilot study, non-parametric tests were employed as they do not assume a normal distribution of the data.

To evaluate differences in protein expression (H -scores) between groups (PV+ vs. PV– and cutaneous vs. oral samples), the Mann–Whitney U test was applied. Potential biological relationships and co-expression patterns between the investigated markers were assessed using the Spearman's rank correlation coefficient, and the corresponding correlation matrix was visualized as a heatmap generated with GraphPad Prism v10.6.1.892.

Descriptive statistics are presented as mean $\pm$ standard deviation (SD). For all analyses, a p -value of $p < 0.05$ was considered statistically significant. Due to the limited cohort size, results where $p > 0.05$ but showing visible differences in distribution were interpreted as trends rather than definitive conclusions.

3 Results

3.1 Samples

Nine out of the 10 samples collected were from females and 1 from a male. The majority of patients ($n = 8$) were of the European breed, with two cases belonging to the Persian breed. All skin samples collected were from ear formations ($n = 7$), while oral samples were from portions of the oral mucosa ($n = 2$), and tongue ($n = 1$).

Regarding the age, the cats were classified according to well-known life stages: kitten (up to 1 year), young adult (1–6 years),

mature adult (7–10), senior (more than 10 years) (22). The predominant cats age diagnosed with neoplastic diseases was senior ($n = 4$), followed by young adult ($n = 5$) and mature adult ($n = 1$) (Table 1).

3.2 Histopathology

The primary lesion observed was consisting in a moderately differentiated SCC, featuring a diffuse, irregular, cord-like infiltrate penetrating the deep dermis. The presence of numerous mitotic rates and multinucleated tumor cells is indicative of elevated proliferative activity. Keratin pearls confirm squamous differentiation, while koilocytes presence indicates a potential role of PV in tumorigenesis (Figure 1A). These architectural and cytologic traits collectively support a diagnosis of SCC.

3.3 PCR

Following the PCR assay, 6 out of 10 (60%) samples were found positive for PV infection. Among these, 5 were from female cats and one from a male. Regarding the breed, 2 samples were from Persian cats, while 4 from European cats. PCR positive samples were from both oral mucosa (2/3; 67%) and skin tissue (4/7; 57%) (Table 1).

3.4 Immunohistochemistry

To explore the reactivity of MMP-2/-9/-13/-14, TIMP-2/-3 and CK10 on tumor cells of feline SCC, the immunostaining was performed as follows: MMP-2 was performed on 10 samples, MMP-9 on 8 samples, MMP-13/-14, TIMP-2/-3 on 9 samples and CK10 on 5 samples.

The distribution and intensity of the immunostaining were consistent for the majority of analyzed samples, reflecting a predominantly cytoplasmic localization. Most samples exhibited moderate cytoplasmic immunoreactivity for MMP-2 (Figure 1B), MMP-9 (Figure 1C), MMP-13 (Figure 1D), and MMP-14 (Figure 1E), with a diffuse and granular pattern. In contrast, TIMP-2 (Figure 1F), and CK10 (Figure 1H) showed weaker cytoplasmic staining often limited to scattered cells, while TIMP-3 displayed an intense cytoplasmic immunoreactivity (Figure 1G). Nuclear staining was generally faint for MMP-2 and MMP-13, moderate for MMP-9, MMP-14, and TIMP-3, and absent for TIMP-2 and CK10.

TABLE 1 Clinical data and laboratory results obtained on the tested samples.

Sample	Age	Sex	Breed	Tumor location	Histopathology	PCR
CSCC1	1 year	F	European	Ear skin	CSCC	–
CSCC2	3 years	F	European	Ear skin	CSCC	+
CSCC3	5 years	F	European	Ear skin	CSCC	+
CSCC4	11 years	F	European	Ear skin	CSCC	–
CSCC5	11 years	F	Persian	Ear skin	CSCC	–
CSCC6	14 years	F	European	Ear skin	CSCC	+
CSCC7	5 years	F	European	Ear skin	CSCC	+
FOSCC1	8 years	F	European	Oral mucosa	OSCC	–
FOSCC2	12 years	F	Persian	Tongue	OSCC	+
FOSCC3	2 years	M	European	Oral mucosa	OSCC	+

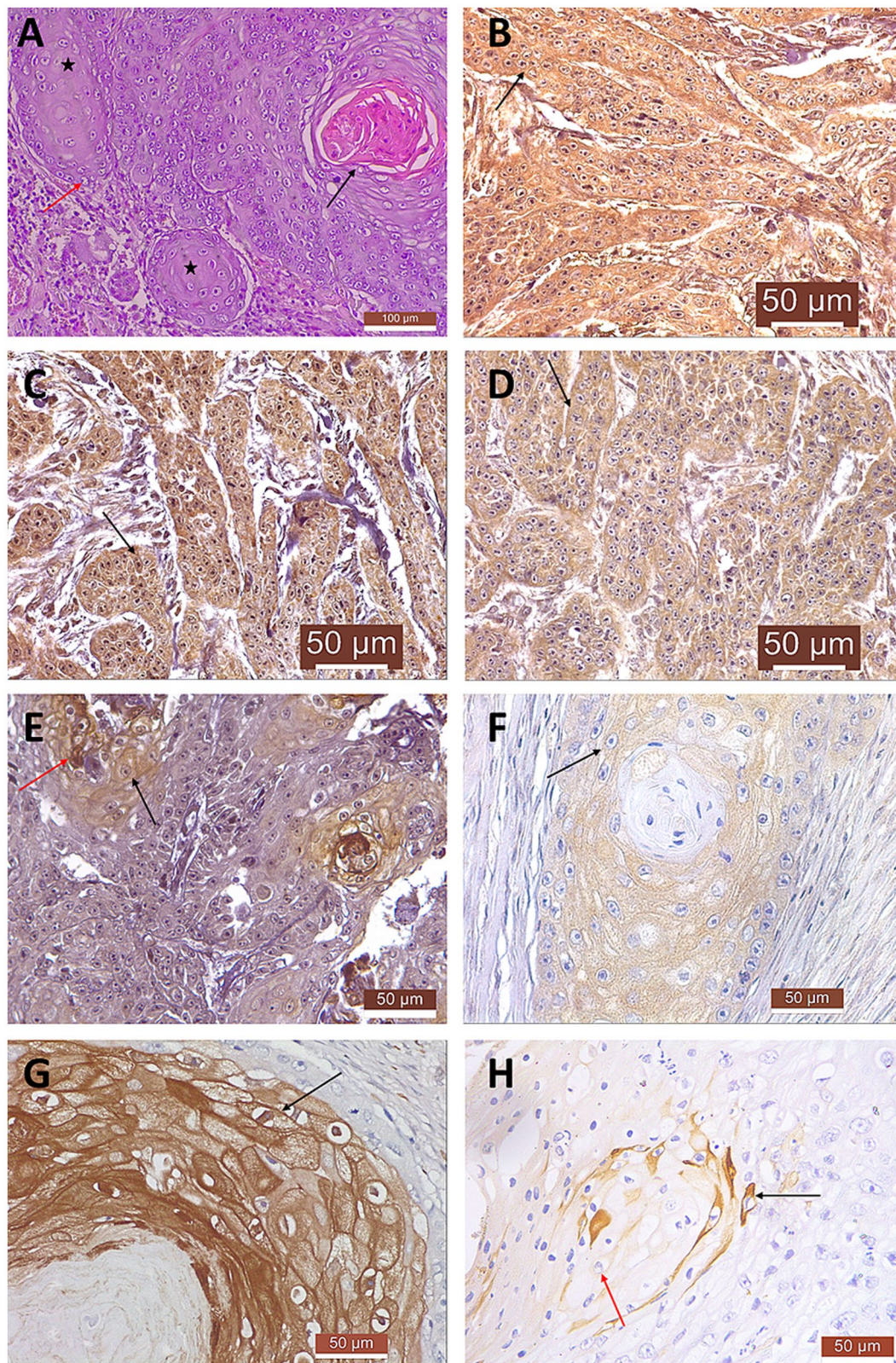


FIGURE 1

Histopathological characterization **(A)** and immunostaining of feline SCC **(B–H)**. **(A)** Moderately differentiated carcinoma exhibiting a diffuse, irregular cord-like infiltration within the deeper dermal layers (black stars), along with the presence of koilocytes (red arrow) and keratin pearl formation (black arrow); HE, 20X; **(B)** MMP-2 immunostaining showing moderate cytoplasmic and weak nuclear pattern (black arrow); 40X. **(C)** MMP-9 immunostaining appears as moderate cytoplasmic and nuclear pattern (black arrow); 40X. **(D)** MMP-13 immunostaining appears as moderate cytoplasmic and weak nuclear pattern (black arrow); 40X. **(E)** MMP-14 immunostaining is observed as moderate cytoplasmic and nuclear (black arrow) to intense cytoplasmic pattern (red arrow); 40X. **(F)** TIMP-2 immunostaining appears as weak to moderate granular cytoplasmic pattern (black arrow); 40X. **(G)** TIMP-3 immunostaining showing moderate to intense cytoplasmic and nuclear pattern (black arrow); 40X. **(H)** CK10 immunostaining appears as weak cytoplasmic (red arrow) to intense cytoplasmic pattern (black arrow); 40X.

H-score analysis using Mann Whitney *U* test indicated a higher expression of MMP-9, TIMP-3, and CK10 in cutaneous samples compared to oral ones, although these differences were not statistically significant (Supplementary Figures 1, 2). The only statistically significant difference was consisting in a higher TIMP-2 expression in PV-negative compared to positive samples.

Spearman's rank correlation analysis (ρ) revealed significant biological relationships between protein expression evaluated by *H*-score (Supplementary Figure 3). Two significant positive correlation were observed between MMP-9 and TIMP-3 ($\rho = 0.81$, $p = 0.015$) and between MMP-2 and MMP-9 ($\rho = 0.71$, $p = 0.046$). The most notable inverse correlation was identified between MMP-2 and CK10 ($\rho = -0.90$, $p = 0.037$).

3.5 Western blot

Western Blot analysis was performed on 5 samples of SCC, 2 from skin and 3 from the oral cavity. Of these, 3 samples were positive for PV, while 2 samples were negative.

Bands corresponding to expected molecular weights were detected for all tested antibodies—MMP-2 (~72 kDa), MMP-9 (~95/60 kDa pro/active forms), MMP-13 (~50 kDa), MMP-14 (~60 kDa) (Figure 2), TIMP-2 (~25 kDa), TIMP-3 (~25 kDa), and CK10 (~55 kDa) (Figure 3), confirming the specificity of IHC staining.

Densitometric analysis suggested possible trends, but was not pursued statistically due to the small sample size. When compared to the cutaneous samples, the oral samples showed higher expression for all investigated proteins. For the analyzed proteins, minor variations could be observed according to PV status, however this variation was not further analyzed due to limited samples (Figures 2, 3).

4 Discussion

This study investigated the expression of MMP-2/-9/-13/-14, TIMP-2/-3, and CK10 antibodies in feline SCC tissue samples.

OSCC is recognized as the most prevalent malignant tumor within the oral cavity of cats, accounting for approximately 60 to 75% of all oral tumors (23, 24). On the other hand, CSCC is one of the most frequently diagnosed skin tumors in felines, accounting for approximately 15–25% of all cutaneous neoplasms in cats (25).

CSCC is notably associated with environmental factors such as ultraviolet (UV) light exposure, particularly in non-pigmented areas of the skin (26). The presence of PV DNA in some cases of feline CSCC further emphasizes the role of viral infections as contributing factors. Research indicates that increased p16 protein expression correlates with UV protection in CSCC, suggesting a complex interaction between viral infections and UV exposure in tumor progression (27, 28). Conversely, feline OSCC displays different biological characteristics and is less likely to be associated with UV exposure. OSCC has been associated with viral infections such as feline PV (29). In our study, PV DNA was identified in 6 of 10 feline SCC cases, both skin and oral samples. This result reinforces the potential role of PV in the development of feline SCC. Future research should focus on identifying the specific feline PV types involved in these tumors.

To the authors knowledge, this is the first study to evaluate the expression of a panel of MMPs, TIMPs, and CK10 in feline SCC tissue samples using immunohistochemistry. Our findings revealed

cytoplasmic MMP-2/-9/-13 and -14 staining in neoplastic cells, consistently with observations reported in human OSCC (30–32). This cytoplasmic presence is consistent with the classical biology of MMPs, reflecting their active synthesis in the form of pro-enzymes (zymogens) and storage in the vesicles of the Golgi apparatus and endoplasmic reticulum before being secreted into the extracellular environment for matrix degradation (33).

Our results are also in accordance with the findings reported by Daraban Bocaneti et al. (11, 12), describing cytoplasmic staining on tumor cells of bovine fibropapilloma using an anti-TIMP-2 and anti-TIMP-3 antibodies. This consistent cytoplasmic localization across different species and tumor types reinforces the role of TIMPs as key intrinsic regulators of the tumor microenvironment.

Furthermore, we identified nuclear reactivity for MMP-9, MMP-14, and TIMP-3, an observation that highlights the roles of these proteins in tumor progression. de Almeida et al. (34) emphasize that the nuclear translocation of MMPs is associated with functions independent of their extracellular proteolytic activity, such as transcriptional regulation, cell cycle control, and influencing genomic stability. Therefore, the nuclear localization of MMP-9 and MMP-14 may suggest a direct involvement in the genetic reprogramming of feline SCC tumor cells. Similarly, the presence of TIMP-3 in the nucleus has been documented to play a role in the regulation of apoptosis and cell survival, providing new insight into the mechanisms of intracellular control in SCC (35).

The strictly cytoplasmic localization of CK10 confirms its structural role as an intermediate filament, maintaining the integrity of the epithelial cytoskeleton without involvement in nuclear signaling (36).

The *H*-score is a quantitative measure to evaluate the expression levels of proteins or antigens in tissue samples. This scoring system integrates both the intensity of staining and the percentage of positively stained cells, providing a more comprehensive assessment than simple percentage scores alone (16). Following *H*-score analysis using Mann–Whitney *U* test, the only statistically significant result was noted on TIMP-2 expression, which was markedly higher in PV-negative samples compared to positive ones, consistent with da Silva Cardeal et al. (37), who demonstrated that co-expression of HPV-16 E6 and E7 oncoproteins negatively regulates TIMP-2 in human cervical cancer, suggesting possible similar mechanisms of virus-host interaction in the feline model.

Spearman's correlation coefficient indicated a positive correlation between MMP-9 and TIMP-3, which may suggest that in feline SCC there is a compensatory response of the tumor microenvironment, attempting to balance the enzymatic activity of MMP-9 through the secretion of TIMP-3, an observation described in studies conducted on human SCC (38). Furthermore, the positive correlation observed between MMP-2 and MMP-9 seems to support the hypothesis of synergism between the two gelatinases in the process of tumor invasion, similar to the mechanisms described in human pathology (39). As for the inverse relationship between MMP-2 and CK10, it could reflect an association between invasive potential and loss of cellular differentiation, suggesting that reduced structural cohesion could facilitate the degradation of the extracellular matrix (ECM) (15). However, given the small size of the cohort, these correlations should be interpreted as biological trends that require validation on a larger sample study.

We acknowledge that the small and heterogeneous cohort, along with the varying number of samples available for certain markers (CK10), was primarily due to the limited quantity of available biological material within the archival blocks. These constraints are

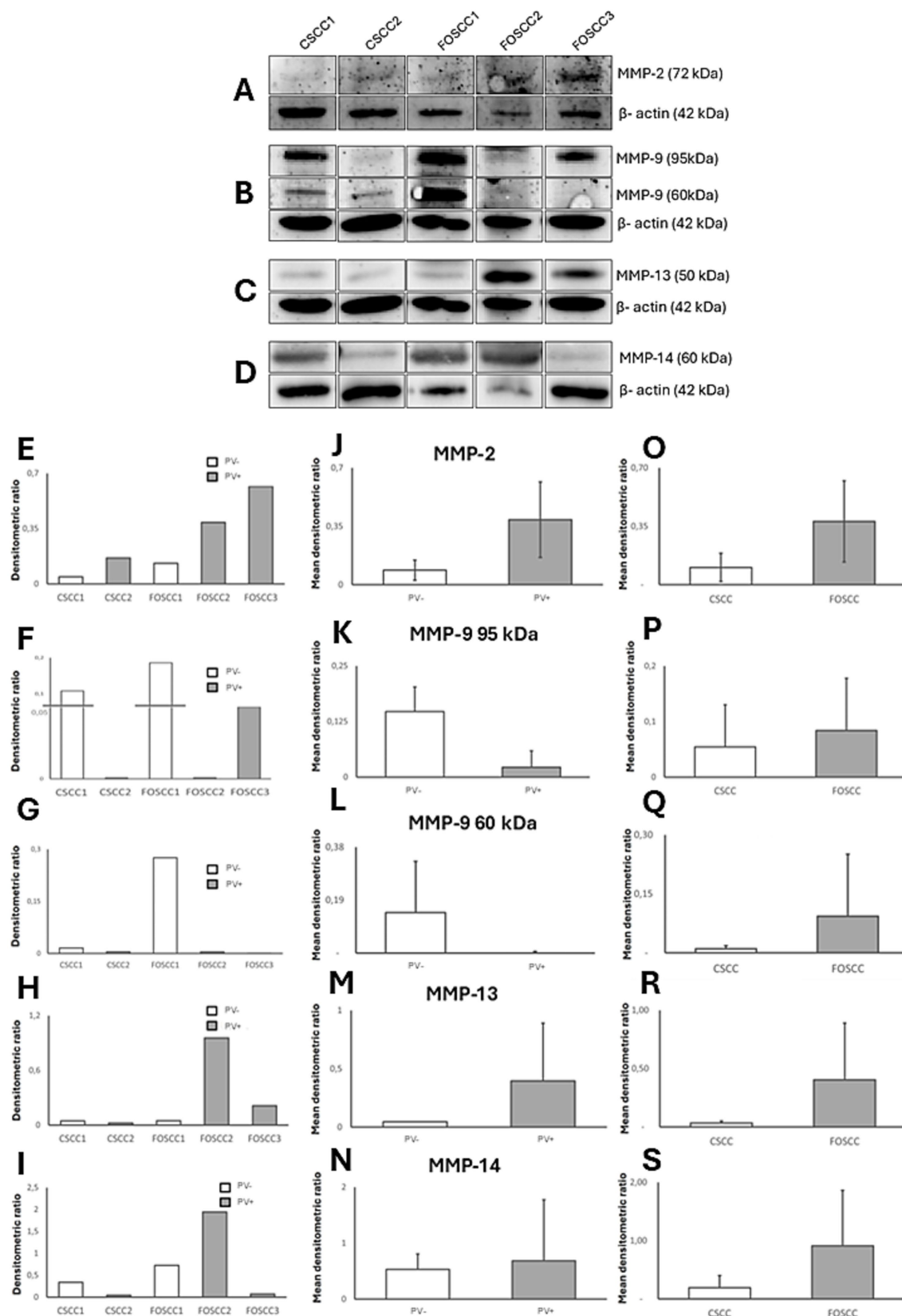


FIGURE 2

Western blotting analysis of MMP-2/-9/-13/-14 in papillomavirus positive (PV+) and papillomavirus negative (PV-) cutaneous squamous cell carcinoma (CSCC) and feline oral squamous cell carcinoma (FOSCC). (A–S) Western blotting and densitometric measurements of MMP-2/-9/-13/-14 in CSCC 1, 2 and FOSCC 1–3 samples. (A–D) Representative gels showing expression of MMP-2/-9/-13/-14. The blots were stripped and tested with anti- β -actin antibody to confirm comparable loading of proteins in each lane and allow normalization. (E–I) Individual densitometric values of MMP-2/-9/-13/-14 for each sample expressed as densitometric ratio with β -actin. (J–N) Mean densitometric values $\pm$ standard deviations between PV+ and PV- samples. (O–S) Mean densitometric values $\pm$ standard deviations between cutaneous and oral samples.

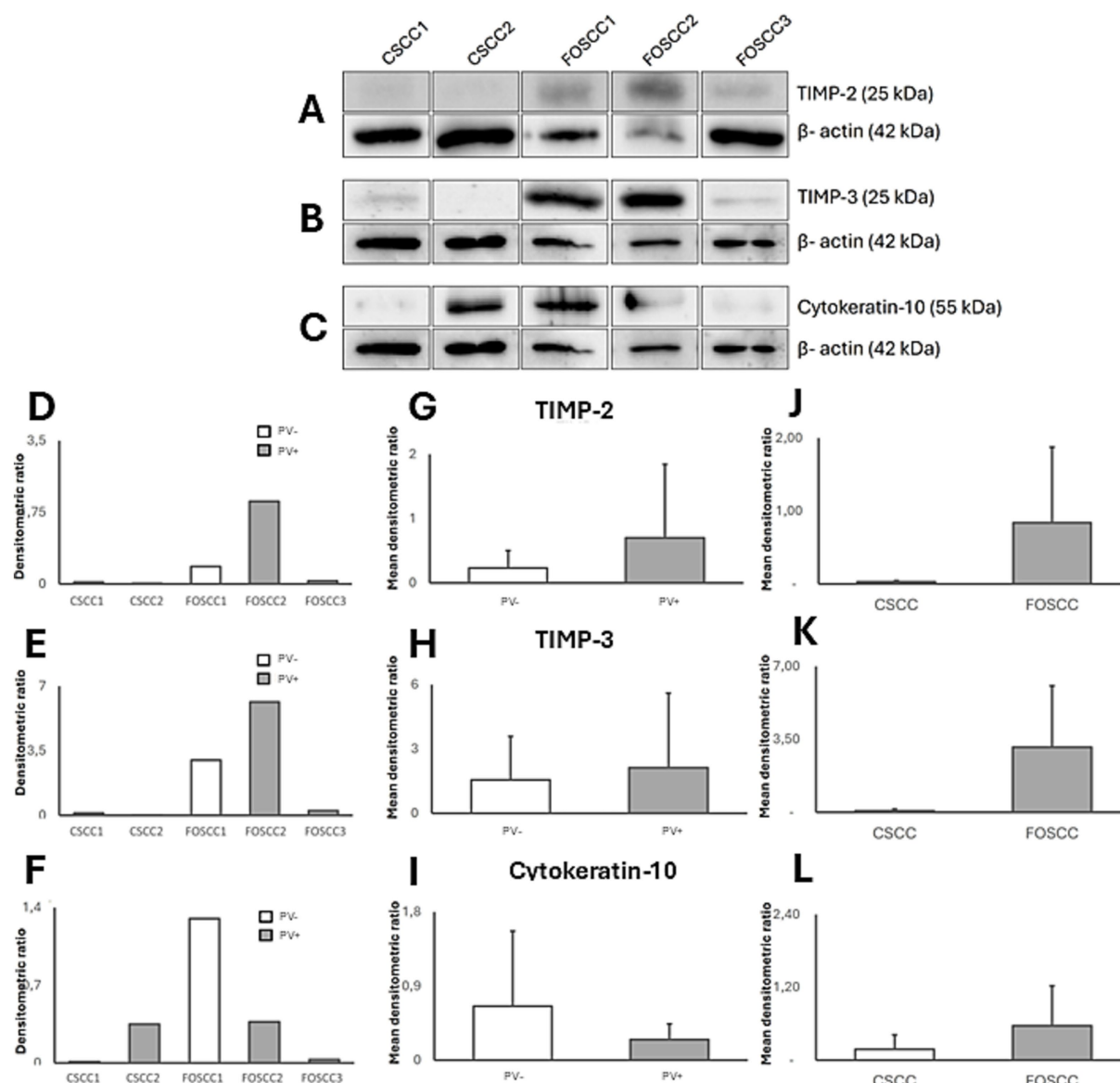


FIGURE 3

Western blotting analysis of TIMP-2/-3 and CK10 in papillomavirus positive (PV+) and papillomavirus negative (PV-) cutaneous squamous cell carcinoma (CSCC) and feline oral squamous cell carcinoma (FOSCC). (A–L) Western blotting and densitometric measurements of TIMP-2/-3 and CK10 in CSCC 1, 2 and FOSCC 1, 2, and 3 samples. (A–C) Representative gels showing expression of TIMP-2/-3 and CK10. The blots were stripped and tested with anti-β-actin antibody to confirm comparable loading of proteins in each lane and allow normalization. (D–F) Individual densitometric values of TIMP-2/-3 and CK10 for each sample expressed as densitometric ratio with β-actin. (G–I) Mean densitometric values ± standard deviations between PV+ and PV- samples. (J–L) Mean densitometric values ± standard deviations between cutaneous and oral samples.

often inherent to investigations utilizing archival diagnostic specimens. The strong correlations observed, despite the limited sample size, provide a robust rationale for further validation in larger, multi-center cohorts.

Western blotting confirmed the specificity of the antibodies used, as bands at the expected molecular weights were detected for all markers tested. Densitometric analysis was performed, yielding results that indicated potential over-expression patterns of MMP-2, -9, -13, -14, and TIMP-2, -3 in feline OSCC versus CSCC. However, due to the limited sample size, these results were not subjected to statistical analysis. The observed patterns are consistent with prior research findings regarding the expression of aforementioned MMP and TIMP expression in oral compared to cutaneous SCC (40, 41). This validation, although performed on a pilot scale, reinforces the IHC observations and supports the translational relevance of the

feline model, aligning with the complex molecular signatures documented in HHNSCC.

The limited sample size prevents generalization of these findings. Future studies involving larger sample populations approaches are needed to validate and reinforce these results, potentially elucidating the role of these markers in feline SCC pathogenesis. Specifically, integrated molecular approaches are necessary to validate these results and to clarify how specific feline PV species modulate tumor invasiveness and differentiation.

In conclusion, this pilot study is the first to characterize the expression of this specific subset of MMPs, TIMPs, and CK10 in feline SCC, integrating IHC and Western blot validation. These results establish a foundation for understanding the viral-molecular axis in feline oncology and reinforce the value of the feline model in comparative oncology research.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by Ethics Committee (Faculty of Veterinary Medicine, IULS, Iasi, Romania). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

PT: Formal analysis, Writing – original draft, Investigation, Writing – review & editing, Conceptualization, Methodology. GA: Formal analysis, Conceptualization, Writing – review & editing, Methodology, Investigation, Writing – original draft. FD: Formal analysis, Writing – original draft, Methodology, Investigation, Supervision, Writing – review & editing, Conceptualization. OH: Writing – review & editing, Investigation, Writing – original draft, Formal analysis. A-SP: Writing – review & editing, Formal analysis, Writing – original draft, Investigation. MD: Investigation, Writing – review & editing, Formal analysis, Writing – original draft. LH: Writing – original draft, Writing – review & editing, Investigation, Formal analysis, Methodology. OT: Writing – review & editing, Investigation, Writing – original draft, Formal analysis. MM: Methodology, Formal analysis, Investigation, Funding acquisition, Writing – review & editing, Conceptualization, Writing – original draft. GB: Writing – review & editing, Conceptualization, Writing – original draft, Supervision.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The financial support was received for the publication of this article from IULS.

References

- Altamura G, Cardeti G, Cersini A, Eleni C, Cocumelli C, del Bartolomé Pino LE, et al. Detection of *Felis catus* papillomavirus type-2 DNA and viral gene expression suggest active infection in feline oral squamous cell carcinoma. *Vet Comp Oncol.* (2020) 18:494–501. doi: 10.1111/vco.12569
- Altamura G, Cuccaro B, Eleni C, Strohmayer C, Brandt S, Borzacchiello G. Investigation of multiple *Felis catus* papillomavirus types (-1/-2/-3/-4/-5/-6) DNAs in feline oral squamous cell carcinoma: a multicentric study. *J Vet Med Sci.* (2022) 84:881–4. doi: 10.1292/JVMS.22-0060
- Munday JS, Dunowska M, De Grey S. Detection of two different papillomaviruses within a feline cutaneous squamous cell carcinoma: case report and review of the literature. *N Z Vet J.* (2011) 57:248–51. doi: 10.1080/00480169.2009.36911
- Foo M, Peura A, Toh YS, Gramer I. Electrochemotherapy is effective in the treatment of early-stage feline cutaneous squamous cell carcinoma. *J Feline Med Surg.* (2025) 27:1098612X251347152. doi: 10.1177/1098612X251347152
- Tozon N, Pavlin D, Sersa G, Dolinsek T, Cemazar M. Electrochemotherapy with intravenous bleomycin injection: an observational study in superficial squamous cell carcinoma in cats. *J Feline Med Surg.* (2014) 16:291–9. doi: 10.1177/1098612X13507071
- Renzi A, Morandi L, Lenzi J, Rigillo A, Bettini G, Bellei E, et al. Analysis of DNA methylation and TP53 mutational status for differentiating feline oral squamous cell carcinoma from non-neoplastic mucosa: a preliminary study. *Vet Comp Oncol.* (2020) 18:825–37. doi: 10.1111/vco.12624
- Rodney AR, Skidmore ZL, Grenier JK, Griffith OL, Miller AD, Chu S, et al. Genomic landscape and gene expression profiles of feline oral squamous cell carcinoma. *Front Vet Sci.* (2023) 10:1079019. doi: 10.3389/FVETS.2023.1079019
- Riedl A-C, Jensen KC, Schirl K, Hoffmann I, Schwinn J, Klopffleisch R, et al. Squamous cell carcinomas in cats: a retrospective study of 4300 histopathological cases (2017–2023). *Pets.* (2026) 3:3. doi: 10.3390/pets3010003

Acknowledgments

The authors wish to thank lab technician Ioana Achirei for her assistance in preparing the histopathological slides.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that Generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2026.1787600/full#supplementary-material>

9. Kaczorowska A, Miękus N, Stefanowicz J, Adamkiewicz-Drożyńska E. Selected matrix metalloproteinases (MMP-2, MMP-7) and their inhibitor (TIMP-2) in adult and pediatric cancer. *Diagnostics*. (2020) 10:547. doi: 10.3390/DIAGNOSTICS10080547
10. Mroczko B, Łukaszewicz-Zajac M, Gryko M, Kedra B, Szmikowski M. Clinical significance of serum levels of matrix metalloproteinase 2 (MMP-2) and its tissue inhibitor (TIMP-2) in gastric cancer. *Folia Histochem Cytobiol*. (2011) 49:125–31. doi: 10.5603/FHC.2011.0018
11. Daraban Bocaneti F, Altamura G, Corteggio A, Tanase OI, Dascau MA, Pasca SA, et al. Expression of matrix metalloproteinases (MMPs)-2/-7/-9/-14 and tissue inhibitors of MMPs (TIMPs)-1/-2 in bovine cutaneous fibropapillomas associated with BPV-2 infection. *Front Vet Sci*. (2022) 9:1063580. doi: 10.3389/fvets.2022.1063580
12. Daraban Bocaneti F, Altamura G, Corteggio A, Tanase OI, Dascau MA, Pasca SA, et al. Expression of collagenases (matrix metalloproteinase-1, -8, -13) and tissue inhibitor of metalloproteinase-3 (TIMP-3) in naturally occurring bovine cutaneous fibropapillomas. *Front Vet Sci*. (2023) 9:1072672. doi: 10.3389/fvets.2022.1072672
13. Ali AA, Al-Jandan BA, Suresh CS. The importance of ckeratins in the early detection of oral squamous cell carcinoma. *J Oral Maxillofac Pathol*. (2018) 22:441. doi: 10.4103/JOMFP.JOMFP_238_17
14. Yang X-R, Xu Y, Shi GM, Fan J, Zhou J, Ji Y, et al. Cytokeratin 10 and cytokeratin 19: predictive markers for poor prognosis in hepatocellular carcinoma patients after curative resection. *Clin Cancer Res*. (2008) 14:3850–9. doi: 10.1158/1078-0432.CCR-07-4338
15. Bachmeier BE, Nerlich AG. Immunohistochemical pattern of cytokeratins and MMPs in human keratinocyte cell lines of different biological behaviour. *Int J Oncol*. (2002) 20:495–9. doi: 10.3892/IJO.20.3.495/ABSTRACT
16. Kazemi A, Fathy M, Jahanian A, Khanali J, Ostadi Y, Babajani A, et al. The role of MMPs and TIMPs in regenerative medicine: from pathological ECM remodeling to therapeutic applications. *Biomed Pharmacother*. (2025) 191:118457. doi: 10.1016/j.biopha.2025.118457
17. Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. *J Gen Virol*. (1999) 80:2437–43. doi: 10.1099/0022-1317-80-9-2437
18. Meuten DJ. *Tumors in Domestic Animals. Tumors in Domestic Animals*. Hoboken: Wiley (2016).
19. Qiu Y, Maione F, Capano S, Meda C, Picconi O, Brundu S, et al. HIV protease inhibitors block HPV16-induced murine cervical carcinoma and promote vessel normalization in association with MMP-9 inhibition and TIMP-3 induction. *Mol Cancer Ther*. (2020) 19:2476–89. doi: 10.1158/1535-7163.MCT-20-0055
20. Wen Z, Luo D, Wang S, Rong R, Evers BM, Jia L, et al. Deep learning-based H-score quantification of immunohistochemistry-stained images. *Mod Pathol*. (2023) 37:100398. doi: 10.1016/J.MODPAT.2023.100398
21. Bocaneti F, Altamura G, Corteggio A, Vesescu E, Borzacchiello G. Expression of bcl-2 and p53 in bovine cutaneous fibropapillomas. *Infect Agent Cancer*. (2015) 10:2. doi: 10.1186/1750-9378-10-2
22. Quimby J, Gowland S, Carney HC, Deporter T, Plummer P, Westropp J. 2021 AAHA/AAFP feline life stage guidelines. *J Feline Med Surg*. (2021) 23:211–33. doi: 10.1177/1098612X21993657
23. Renzi A, De Bonis P, Morandi L, Lenzi J, Tinto D, Rigillo A, et al. Prevalence of p53 dysregulations in feline oral squamous cell carcinoma and non-neoplastic oral mucosa. *PLoS One*. (2019) 14:e0215621. doi: 10.1371/JOURNAL.PONE.0215621
24. Wiles V, Hohenhaus A, Lamb K, Zaidi B, Camps-Palau M, Leibman N. Retrospective evaluation of toceranib phosphate (Palladia) in cats with oral squamous cell carcinoma. *J Feline Med Surg*. (2017) 19:185–93. doi: 10.1177/1098612X15622237
25. Ho NT, Smith KC, Dobromylskyj MJ. Retrospective study of more than 9000 feline cutaneous tumours in the UK: 2006–2013. *J Feline Med Surg*. (2018) 20:128–34. doi: 10.1177/1098612X17699477
26. Egberink H, Hartmann K, Mueller R, Pennisi MG, Belák S, Tasker S, et al. Feline papillomatosis. *Viruses*. (2025) 17:59. doi: 10.3390/V17010059
27. Munday JS, Gibson I, French AF. Papillomaviral DNA and increased p16CDKN2A protein are frequently present within feline cutaneous squamous cell carcinomas in ultraviolet-protected skin. *Vet Dermatol*. (2011) 22:360–6. doi: 10.1111/J.1365-3164.2011.00958.X
28. Munday JS, Sharp CR, Beatty JA. Novel viruses: update on the significance of papillomavirus infections in cats. *J Feline Med Surg*. (2019) 21:409–18. doi: 10.1177/1098612X18808105
29. Tutu P, Daraban Bocaneti F, Altamura G, Dascau MA, Horodincu L, Soreanu OD, et al. Feline oral squamous cell carcinoma: recent advances and future perspectives. *Front Vet Sci*. (2025) 12:1663990. doi: 10.3389/fvets.2025.1663990
30. Hong SD, Hong SP, Kim YS, Lee JI, Lim CY. Expression of matrix metalloproteinase-2 and -9 in oral squamous cell carcinomas in relation to the histologic invasiveness and cellular differentiation. *Korean J Pathol*. (1999) 33:243–50.
31. Vincent-Chong VK, Salahshourifar I, Karen-Ng LP, Siow MY, Kallarakkal TG, Ramanathan A, et al. Overexpression of MMP13 is associated with clinical outcomes and poor prognosis in oral squamous cell carcinoma. *Sci World J*. (2014) 2014:897523. doi: 10.1155/2014/897523
32. Rojas YVC, Cruz-Romero SD, Baldiñ AM, Tawil JAH, Baldiñ PA, Rodriguez-Urrego PA. Prognostic significance of MMP-14 in oral squamous cell carcinoma: insights from a Colombian cohort. *Oral Surg Oral Med Oral Pathol Oral Radiol*. (2025) 141:654–66. doi: 10.1016/j.oooo.2025.11.007
33. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. *J Pathol*. (2003) 200:448–64. doi: 10.1002/PATH.1400
34. de Almeida LGN, Thode H, Eslambolchi Y, Chopra S, Young D, Gill S, et al. Matrix metalloproteinases: from molecular mechanisms to physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. (2022) 74:714–70. doi: 10.1124/PHARMREV.121.000349
35. Zhao H, Bernardo MM, Osenkowski P, Sohail A, Pei D, Nagase H, et al. Differential inhibition of membrane type 3 (MT3)-matrix metalloproteinase (MMP) and MT1-MMP by tissue inhibitor of metalloproteinase (TIMP)-2 and TIMP-3 regulates pro-MMP-2 activation. *J Biol Chem*. (2004) 279:8592–601. doi: 10.1074/jbc.M308708200
36. Mykhaliuk VV, Havryliak VV, Salyha YT. The role of cytokeratins in ensuring the basic cellular functions and in diagnosis of disorders. *Cytol Genet*. (2022) 56:530–40. doi: 10.3103/S0095452722060093
37. da Silva Cardeal LB, Boccardo E, Termini L, Rabachini T, Andreoli MA, di Loreto C, et al. HPV16 oncoproteins induce MMPs/RECK-TIMP-2 imbalance in primary keratinocytes: possible implications in cervical carcinogenesis. *PLoS One*. (2012) 7:e33585. doi: 10.1371/JOURNAL.PONE.0033585
38. Wróbel-Roztropiński A, Zielińska-Kaźmierska B, Roztropiński H, Lucas-Grzelczyk W, Szemraj J, Józefowicz-Korczyńska M. Expression of matrix metalloproteinases (MMPs) and their inhibitor (TIMP) genes on mRNA and protein levels in oral squamous cell carcinoma. *Nowotwory J Oncol*. (2021) 71:1–8. doi: 10.5603/NJO.2021.0003
39. Aparna M, Rao L, Kunhikatta V, Radhakrishnan R. The role of MMP-2 and MMP-9 as prognostic markers in the early stages of tongue squamous cell carcinoma. *J Oral Pathol Med*. (2015) 44:345–52. doi: 10.1111/jop.12245
40. Stephens P, Davies KJ, Occlleston N, Pleass RD, Kon C, Daniels J, et al. Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix reorganization and matrix metalloproteinase activity. *Br J Dermatol*. (2001) 144:229–37. doi: 10.1046/J.1365-2133.2001.04006.X
41. Kornfeld JW, Meder S, Wohlberg M, Friedrich RE, Rau T, Riethdorf L, et al. Overexpression of TACE and TIMP3 mRNA in head and neck cancer: association with tumour development and progression. *Br J Cancer*. (2011) 104:138–45. doi: 10.1038/SJ.BJC.606017