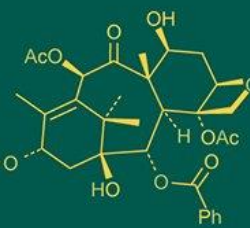
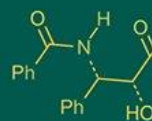


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(5): 628-634
www.biochemjournal.com
 Received: 18-02-2026
 Accepted: 21-03-2026

Pradeep Kumar
 Toxicogenomics Laboratory,
 Department of Animal Science,
 M.J.P. Rohilkhand University,
 Bareilly, Uttar Pradesh, India

Sonal Saxena
 Veterinary Immunology
 Section, ICAR-Indian
 Veterinary Research Institute,
 Izatnagar, Bareilly, Uttar
 Pradesh, India

Abha Trivedi
 Toxicogenomics Laboratory,
 Department of Animal Science,
 M.J.P. Rohilkhand University,
 Bareilly, Uttar Pradesh, India

Sameer Shrivastava
 Veterinary Immunology
 Section, ICAR-Indian
 Veterinary Research Institute,
 Izatnagar, Bareilly, Uttar
 Pradesh, India

Corresponding Author:
Sonal Saxena
 Veterinary Immunology
 Section, ICAR-Indian
 Veterinary Research Institute,
 Izatnagar, Bareilly, Uttar
 Pradesh, India

Molecular cloning and phylogenetic characterisation of beta-2 microglobulin in canine mammary tumours

Pradeep Kumar, Sonal Saxena, Abha Trivedi and Sameer Shrivastava

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5h.8468>

Abstract

Cancer remains a major global health challenge, necessitating the identification of reliable biomarkers for diagnosis and prognosis. β 2-Microglobulin (B2M), a key component of MHC class I molecules, has been implicated in tumour progression and immune modulation; however, its role in canine mammary tumours (CMTs) remains poorly understood. The present study aimed to evaluate the expression, molecular characterisation, and functional relevance of B2M in CMTs. Tumour and control samples were subjected to histopathological examination, immunohistochemistry (IHC), gene amplification, cloning, protein expression, and phylogenetic analysis. Histopathological findings revealed predominantly malignant tumours, including tubular, tubulopapillary, complex, and solid carcinomas with moderate to poor differentiation. IHC analysis demonstrated moderate to strong B2M expression in neoplastic mammary epithelial cells, particularly in poorly differentiated tumour regions. Molecular analysis confirmed successful amplification of the B2M gene (288 bp), cloning into pET32 expression vector. Phylogenetic analysis revealed high evolutionary conservation of B2M across species, with *Canis lupus familiaris* clustering closely with related mammals. B2M is associated with tumour progression and histological grade in CMTs. Its conserved molecular characteristics and involvement in tumour biology highlight its potential as a diagnostic and prognostic biomarker, as well as a promising target for translational cancer research.

Keywords: β 2-microglobulin, canine mammary tumour, immunohistochemistry, biomarker, cloning and expression, cancer progression

Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide, posing a major public health challenge. According to recent global estimates, approximately 19.3 million new cancer cases and nearly 10 million cancer-related deaths were reported in 2020, with the burden expected to rise to over 28 million new cases annually by 2040 due to population growth, aging, and lifestyle changes (Sung *et al.*, 2021; Ferlay *et al.*, 2021) [14, 3]. Among all cancer types, breast, lung, colorectal, and prostate cancers account for a significant proportion of global incidence and mortality. In addition, disparities in cancer burden are evident between developed and developing countries, where limited access to early diagnosis and treatment contributes to poorer outcomes (Bray *et al.*, 2018) [2]. β 2-Microglobulin (B2M) is a low molecular weight (~12 kDa), non-glycosylated protein that constitutes the light chain of major histocompatibility complex class I (MHC-I) molecules and is expressed on the surface of all nucleated cells. It plays a crucial role in antigen presentation and immune surveillance by stabilising MHC-I complexes and facilitating the recognition of intracellular antigens by CD8⁺ T lymphocytes (Jackson *et al.*, 1999; Ploegh, 1988) [7, 9]. Under normal physiological conditions, B2M is continuously shed into circulation, and its serum concentration reflects cellular turnover and immune system activity, making it a useful biomarker in various pathological conditions, including cancer (Prizment *et al.*, 2016) [10].

In human cancers, B2M has been extensively studied as both a diagnostic and prognostic biomarker. Elevated levels of circulating B2M have been reported in haematological malignancies such as multiple myeloma, lymphoma, and leukaemia, as well as in solid tumours including breast, lung, prostate, and renal cancers. Increased B2M levels are strongly associated with tumour burden, disease progression, and poor clinical outcomes (Bataille & Klein, 1992; Zhang *et al.*, 2013) [1, 15].

In addition to its role as a biomarker, B2M actively contributes to tumour progression by modulating signalling pathways involved in proliferation, apoptosis, angiogenesis, and metastasis. For instance, B2M has been shown to activate pathways such as protein kinase A/CREB and PI3K/Akt, thereby promoting cancer cell survival and growth (Huang *et al.*, 2006)^[6]. Conversely, loss or mutation of B2M can impair MHC-I expression, leading to reduced antigen presentation and enabling tumour immune evasion, which contributes to resistance to immunotherapy (Han *et al.*, 2025; Reis *et al.*, 2024)^[5, 12].

Comparative oncology offers a valuable approach to understanding cancer biology across species, particularly between dogs and humans. Canine cancers develop spontaneously and share significant similarities with human malignancies in terms of histopathology, molecular pathways, tumour microenvironment, and clinical progression. Among these, canine mammary tumours (CMTs) are one of the most common neoplasms in female dogs and closely resemble human breast cancer, making them an important translational model (Queiroga & Lopes, 2011; Nosalova *et al.*, 2024)^[11, 8].

2. Materials and Methods

2.1 Collection of serum and tissue samples

Serum and tissue samples were collected from suspected cases of canine mammary gland tumours (CMTs) in female dogs presented at the Referral Veterinary Polyclinic, ICAR-IVRI-Izatnagar, Bareilly. Clinically healthy female dogs served as controls. Tumour dimensions were measured using Vernier callipers, and blood samples from the cephalic vein were processed to obtain serum, which was stored at -80°C until use. The study was conducted with the approval of the Institute Animal Ethics Committee (IAEC), ICAR-IVRI (Approval No. F.26-1/2015-16/JD.R, dated 28th February 2019; Approval No. 26-1/2023-24/JD(R)/IAEC, dated 7th November 2023), in accordance with the Government of India's Amendment Rules for the Control and Supervision of Experiments on Animals (CPCSEA), 2005.

2.2 Histopathological examination

Tumour and adjacent normal mammary tissues were aseptically collected from clinically diagnosed canine cases during surgical excision, rinsed in phosphate-buffered saline (PBS), and fixed in 10% neutral buffered formalin for 24-48 h. Fixed tissues were processed using standard histological procedures, including dehydration through graded ethanol, xylene clearing, and paraffin embedding. Sections (4-5 μm) were prepared using a rotary microtome, mounted on poly-L-lysine coated slides, and dried at 60°C . For staining, sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). Slides were then dehydrated, cleared, and mounted with DPX. Histopathological evaluation was performed under a light microscope at different magnifications (10 $\times$, 40 $\times$, and 100 $\times$) based on cellular morphology, differentiation, mitotic activity, nuclear pleomorphism, and stromal invasion. Tumours were classified and graded according to established criteria (Goldschmidt *et al.*, 2011; Sorenmo *et al.*, 2009)^[4, 13].

2.3 Immunohistochemistry for $\beta 2$ -microglobulin

Paraffin-embedded tissue sections (4-5 μm) were mounted on poly-L-lysine (Sigma-Aldrich) coated slides and

incubated at 60°C for 1 h. Sections were deparaffinized in xylene (Merk) and rehydrated through graded ethanol to distilled water. Antigen retrieval was performed in a citrate buffer (10 mM, pH 6.0; Abcam) in a microwave oven for 10-15 min, then cooled at room temperature. Endogenous peroxidase activity was blocked by incubating sections with 3% hydrogen peroxide (Sigma-Aldrich) for 10 min. Non-specific binding was minimised by incubating the sections with normal serum or blocking buffer for 20 min. The sections were then incubated overnight at 4°C with a polyclonal anti- $\beta 2$ -M antibody (1:50; Thermo Fisher Scientific, PA5-87248; in 2.5% BSA). After washing with PBS, sections were incubated with HRP-conjugated anti-dog secondary antibody (Sigma-Aldrich, 1:200) for 60 min at room temperature. Immunoreactivity was visualised using 3,3'-diaminobenzidine (DAB) as the chromogen, resulting in a brown precipitate at antigen sites. Sections were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted with DPX.

2.4 RNA isolation and cDNA synthesis

Total RNA was extracted from tumour and control tissue samples using TRIzol® reagent (Invitrogen, USA) following the manufacturer's protocol. The concentration and purity of isolated RNA were determined using a NanoDrop™ spectrophotometer (Thermo-Fisher Scientific, USA) by measuring absorbance at 260/280 nm. RNA integrity was confirmed by electrophoresis on a 1.5% agarose gel. For complementary DNA (cDNA) synthesis, 1 μg of total RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo-scientific) in a final reaction volume of 20 μL , according to the manufacturer's instructions. The synthesised cDNA was stored at -20°C until further analysis.

2.5 PCR amplification of the B2M Gene

The canine Beta-2 microglobulin (*B2M*) gene was amplified using gene-specific primers designed from the conserved canine *B2M* coding sequence. Primer design was performed using Primer Premier 5.0 (National Bioscience) and OligoAnalyzer tool 3.1 (Integrated DNA Technologies, USA). PCR amplification was carried out in a thermal cycler using synthesised canine mammary tumour-derived cDNA as template. The reaction mixture consisted of template cDNA, gene-specific forward and reverse primers, Phusion polymerase (Thermo-Scientific), dNTPs (Takara) and nuclease-free water in optimised concentrations. Amplified PCR products were resolved on 1.5% agarose gel electrophoresis and visualised under ultraviolet illumination following nucleic acid staining.

2.6 Cloning of the canine B2M gene

The 50 μL PCR-amplified *B2M* gene products were purified using the MinElute PCR Purification Kit (Qiagen). Purified amplicons were subsequently digested with *KpnI* and *Sall* restriction enzymes (New England Biolabs, USA). Following restriction digestion, the digested products were further purified using a PCR cleanup kit (Qiagen) to remove residual enzymes and reaction components. The purified inserts were then ligated into the pH6HTN His₆ HaloTag PET32 expression vector (Promega) using T4 DNA ligase (Takara Bio Inc., Japan). The recombinant plasmid constructs were transformed into *Escherichia coli* DH5 α competent cells and plated onto Luria-Bertani (LB) agar

plates supplemented with 100 µg/mL ampicillin for selection of positive transformants.

2.7 Phylogenetic analysis

Identification of nucleotide sequences homologous to the isolated B2M gene was performed using BLAST against the NCBI nucleotide database. The retrieved B2M sequences were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm implemented in MEGA 7 software (REF). Phylogenetic relationships were subsequently inferred using the maximum-likelihood method after selection of the best-fit nucleotide substitution model.

3. Results

3.1 Clinical characteristics and sample collection of canine tumour cases

Gross examination of canine mammary tumour cases revealed pronounced heterogeneity in tumour size, shape, and external morphology. The excised tumour mass (Fig. 1A) was typically irregular, firm, and lobulated, with focal areas of congestion and haemorrhage, consistent with active and progressive tumour growth. vernier calliper-based measurements demonstrated substantial tumour dimensions, indicative of advanced tumour development. Preoperative *in vivo* assessment of the affected mammary gland (Fig. 1B).

3.2 Histopathological classification of canine mammary tumours

Histopathological examination of canine mammary tumour sections revealed diverse morphological patterns corresponding to different tumour subtypes. Features of adenosquamous carcinoma, characterized by mixed glandular and squamous differentiation with marked cellular pleomorphism and prominent nucleoli (Fig. 2A). Demonstrated carcinoma in situ, with neoplastic epithelial cells proliferating within well-defined ductal structures, remaining confined by an intact basement membrane, indicating a non-invasive stage of tumour progression (Fig. 2B). Features of comedocarcinoma were observed, including central areas of necrosis within dilated ducts surrounded by malignant epithelial cells, suggestive of aggressive tumour behaviour (Fig. 2C). It exhibited characteristics of complex-type carcinoma, with the presence of both epithelial and myoepithelial components arranged in irregular nests and cords, indicating biphasic tumour morphology (Fig. 2D). It revealed fibrosarcoma, composed of spindle-shaped neoplastic cells arranged in interlacing bundles within a collagen-rich stroma, reflecting mesenchymal tumour differentiation (Fig. 2E). Normal canine mammary gland tissue exhibiting preserved ductal and alveolar architecture with normal epithelial cell morphology, included as a non-neoplastic control for comparative pathological and molecular analyses (Fig. 2F).

3.3 Immunohistochemical expression of β 2-microglobulin

Immunohistochemical analysis demonstrated distinct β 2-microglobulin expression in canine mammary tumour tissues. Representative tumour sections showed positive immunoreactivity characterized by brown chromogenic staining predominantly localized to the cytoplasm and cell membrane of neoplastic mammary epithelial cells. The intensity of staining ranged from moderate to strong and

was more pronounced in densely cellular and poorly differentiated tumour regions, indicating elevated B2M expression in malignant tissues (Fig. 3A-B). The negative control section (Fig. 3C), processed without the primary antibody, exhibited no specific staining, confirming the specificity of the immunohistochemical reaction.

3.4 PCR amplification of canine B2M gene

Successful amplification of the canine *Beta-2 microglobulin* (B2M) gene was achieved from canine mammary tumour-derived cDNA samples using gene-specific primers. Agarose gel electrophoresis analysis revealed distinct and well-defined amplification bands corresponding to the expected amplicon size of 288 bp in lanes L1-L4, confirming efficient amplification of the target B2M gene across all analysed tumour samples (Fig. 4). The amplified B2M PCR products were visualised under ultraviolet illumination following electrophoretic separation on an ethidium bromide-stained 1.5% agarose gel.

3.5 Cloning and molecular confirmation of the canine B2M gene

The PCR-amplified canine B2M gene was successfully cloned into the pET-32b expression vector and transformed into *Escherichia coli* DH5 α competent cells. Numerous bacterial colonies were observed on LB agar plates supplemented with ampicillin, indicating successful transformation and uptake of the recombinant plasmid construct (Fig. 5A).

Restriction digestion analysis of the recombinant plasmid revealed the presence of the linearized pET-32b vector of approximately 5900 bp along with the released B2M insert of 288 bp, confirming successful ligation of the target gene into the expression vector (Fig. 5B). Furthermore, colony PCR screening of transformed DH5 α colonies generated distinct amplification bands corresponding to the expected 288 bp B2M gene fragment (Fig. 5C), thereby confirming the presence of the recombinant construct in positive bacterial colonies. These confirmed recombinant clones were subsequently utilized for downstream molecular characterization and expression studies.

3.5 Phylogenetic analysis of the B2M gene

The phylogenetic analysis of the β 2-microglobulin (B2M) gene revealed clear evolutionary relationships among the analyzed sequences. The B2M sequences clustered into multiple clades, indicating a high degree of conservation with moderate sequence divergence across species. Closely related sequences grouped together within the same clades, supported by varying bootstrap values, confirming the reliability of the observed clustering patterns.

The *Canis lupus familiaris* B2M sequence clustered with homologous mammalian B2M sequences, demonstrating its close evolutionary relationship with other carnivores and higher mammals. Several internal nodes showed moderate to high bootstrap support ($\geq 70\%$), suggesting stable evolutionary lineages, whereas some branches exhibited lower bootstrap values, reflecting minor sequence variations and possible evolutionary divergence.

Overall, the phylogenetic tree indicates that the B2M gene is evolutionarily conserved, with species-specific variations contributing to distinct sub-clustering. These findings support the functional conservation of B2M across species and validate its suitability for comparative and evolutionary

studies, as well as for translational relevance between canine and other mammalian cancer models (Fig. 6).

4. Discussion

The present study provides a comprehensive evaluation of β 2-microglobulin (B2M) in canine mammary tumours (CMTs) through histopathological, immunohistochemical, and molecular approaches, highlighting its potential role as a biomarker and its relevance in comparative oncology. The observed heterogeneity in tumour morphology and size is consistent with previous reports describing the diverse biological behaviour of CMTs, ranging from benign to highly aggressive malignant phenotypes (Queiroga & Lopes, 2011; Sorenmo *et al.*, 2009) [11, 13]. Histopathological analysis revealed that the majority of tumours were malignant, predominantly classified as tubular, tubulopapillary, complex, and solid carcinomas with moderate to poor differentiation (grades II-III). These findings align with earlier studies indicating that higher-grade tumours exhibit increased cellular atypia, mitotic activity, and stromal invasion, which are hallmarks of aggressive tumour behaviour and poor prognosis (Goldschmidt *et al.*, 2011; Nosalova *et al.*, 2024) [4, 8].

The successful amplification and cloning of the canine *B2M* gene further validate the molecular integrity and conserved nature of this gene. The identification of a distinct 288 bp amplicon confirmed the specificity and reliability of the experimental approach employed in the present study. Similar molecular characteristics and conserved expression patterns of *B2M* have been reported across different species, supporting its fundamental biological role in cellular and immune regulatory mechanisms (Jackson *et al.*, 1999) [7]. The phylogenetic analysis demonstrates a high degree of evolutionary conservation of the β 2-microglobulin (B2M) gene across species, indicating its essential and conserved biological function in MHC-I-mediated antigen presentation (Jackson *et al.*, 1999) [7]. This conserved functional role is critical for maintaining immune surveillance and cellular homeostasis. Importantly, the preserved structure and function of B2M across species suggest that its dysregulation may have similar pathological consequences, including tumour progression and immune evasion. In the context of cancer, elevated B2M expression has been linked to activation of oncogenic signalling pathways and resistance to immune-mediated tumour clearance (Huang *et al.*, 2006; Han *et al.*, 2025; Reis *et al.*, 2024) [6, 5, 12].

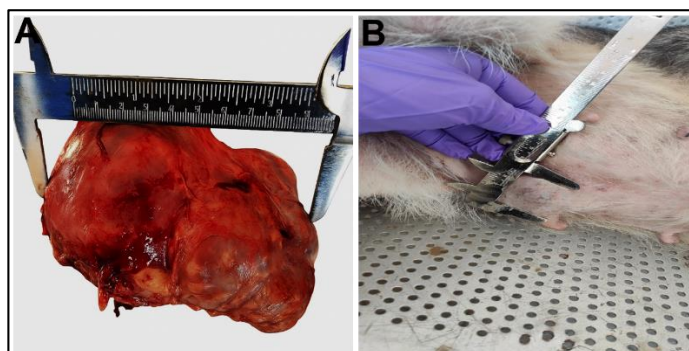


Fig 1: Gross examination and measurement of canine mammary tumours. (A) External appearance of a canine mammary tumour showing visible swelling and skin stretching. (B) Surgical exposure of mammary tumour mass during mastectomy. Pre-surgical tumour size measurement using a vernier calliper. Excised mammary tumour specimens showing variation in size, morphology, and texture. Post-excision tumour mass measurements with callipers to assess tumour dimensions and morphology.

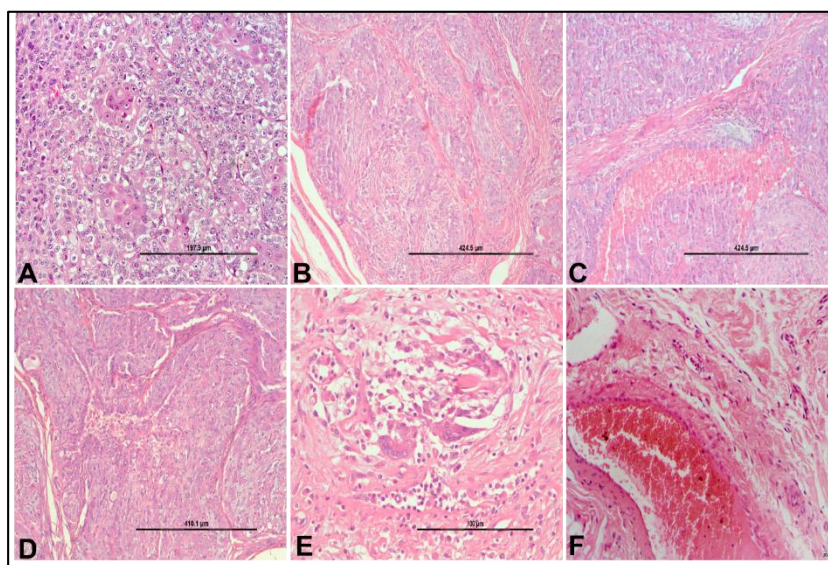


Fig 2: Histopathological features of canine mammary tumours. Representative hematoxylin and eosin (H&E)-stained sections illustrating diverse histological subtypes of canine mammary tumours. (A) Adenosquamous carcinoma showing mixed glandular and squamous differentiation with pleomorphic tumour cells. (B) Carcinoma in situ characterized by neoplastic epithelial proliferation confined within the basement membrane. (C) Comedocarcinoma exhibiting central necrosis within tumour ducts surrounded by malignant epithelial cells. (D) Complex-type carcinoma displaying both epithelial and myoepithelial cell proliferation. (E) Fibrosarcoma composed of spindle-shaped neoplastic cells arranged in interwoven bundles within a collagenous stroma. (F) normal mammary tissue (H&E, $\times 200$).

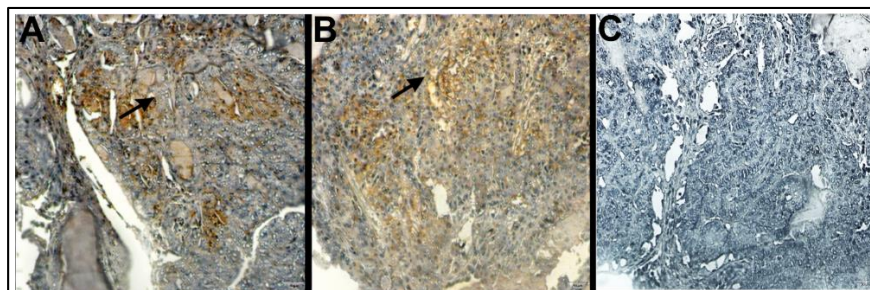


Fig. 3: Immunohistochemical expression of β 2-microglobulin (B2M) in canine mammary tumours. Representative photomicrographs show positive B2M immunoreactivity (brown staining) in neoplastic mammary epithelial cells. (A-B) demonstrate moderate to strong cytoplasmic and membranous staining in tumour tissues (arrows indicate positively stained cells). (C) represents the negative control, showing no specific immunostaining. Sections were counterstained with hematoxylin (H $\times$ 100).

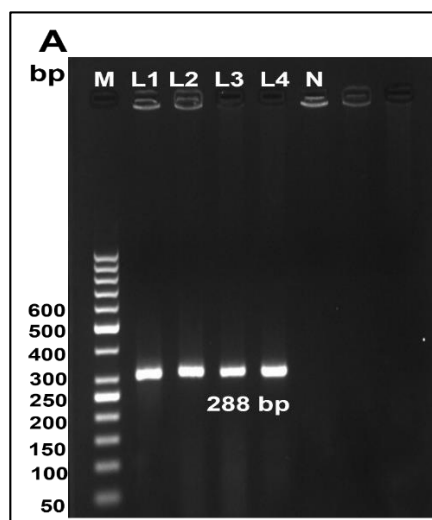


Fig. 4: Agarose gel electrophoresis showing PCR amplification of the canine B2M gene from canine mammary tumour-derived cDNA samples. Lane M: 50 bp DNA molecular weight marker; Lanes L1-L4: amplified B2M gene products showing the expected amplicon size of 288 bp; Lane N: negative control showing no amplification. PCR products were visualised under UV illumination on an ethidium bromide-stained 1.5% agarose gel.

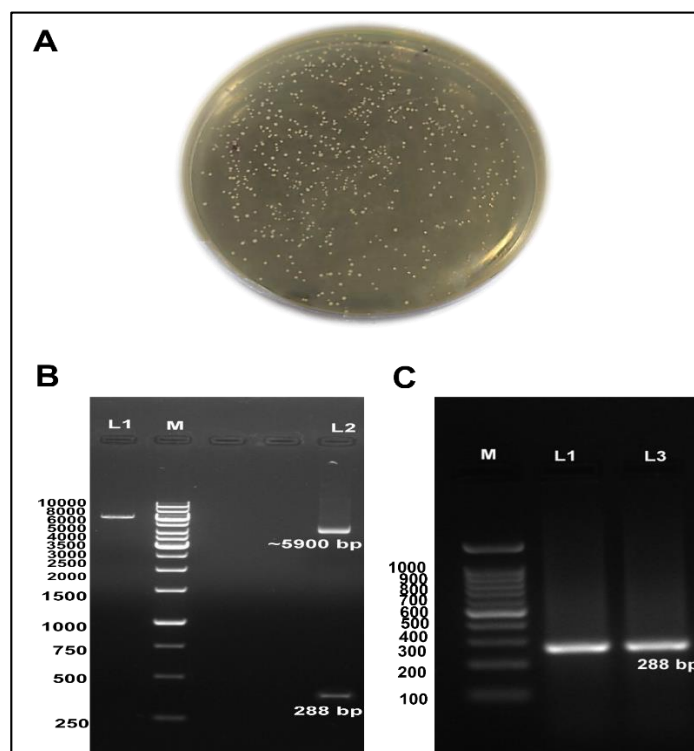


Fig. 5: Cloning and screening of the target gene in the pET-32b expression vector. (A) Representative LB agar plate showing bacterial colonies obtained after transformation, indicating successful uptake of the recombinant plasmid. (B) Agarose gel electrophoresis showing restriction digestion of the recombinant pET-32b plasmid (~5900 bp) and PCR amplification of the target gene (288 bp). (C) Colony PCR analysis of DH5 α competent cells transformed with the pET-32b construct, confirming the presence of the 288 bp insert in positive colonies.

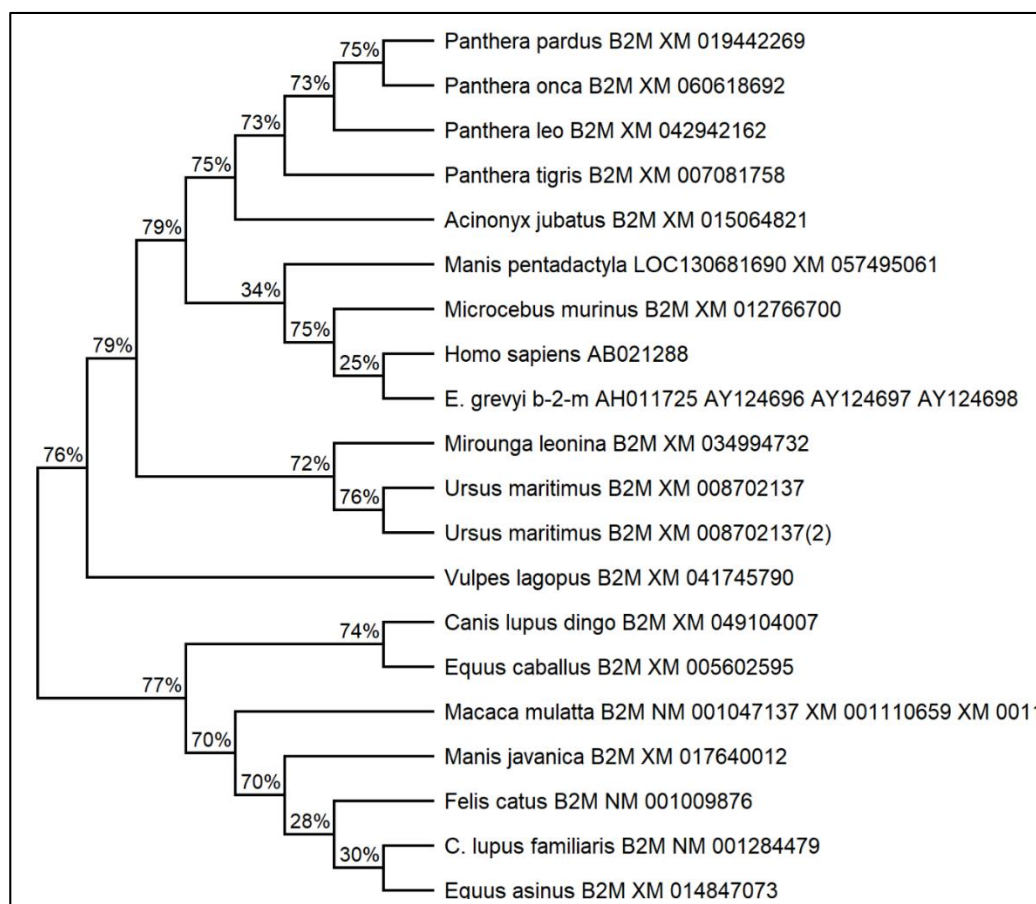


Fig. 6: Phylogenetic analysis of the β 2-microglobulin (B2M) gene. The phylogenetic tree was constructed based on nucleotide sequences of the B2M gene from *Canis lupus familiaris* and other representative species retrieved from the NCBI database. Sequence alignment was performed before tree construction, and the evolutionary relationships were inferred using the Neighbour-Joining method. Bootstrap analysis with 1000 replicates was carried out to assess the robustness of the tree, and bootstrap values (percentage support) are shown at the corresponding nodes. The scale bar represents the number of nucleotide substitutions per site.

Conclusion

β 2-Microglobulin (B2M) is significantly associated with tumour progression and higher histological grades and immunohistochemical analysis in canine mammary tumours, indicating its potential as a diagnostic and prognostic biomarker. Its elevated expression in malignant tissues and conserved molecular characteristics highlight its functional role in tumour biology. Phylogenetic analysis further supports its evolutionary conservation and translational relevance. These findings suggest that B2M may serve as a promising target for future cancer diagnostics and therapeutic strategies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to the Director, ICAR-IVRI, Izatnagar, for providing the necessary funding and support. The study was conducted with support from Grants received under ICAR LAL Bahadur Shastri Outstanding Young Scientist Award Project, (F.no 5-4/2022-A.C (e.21930) and Advanced Research Project on Canines.

References

1. Bataille R, Klein B. Prognostic factors in multiple myeloma. *Blood*. 1992;80(3):733-737.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide. *CA Cancer J Clin*. 2018;68(6):394-424.
3. Ferlay J, Colombet M, Soerjomataram I, *et al*. Estimating the global cancer incidence and mortality in 2018 and 2020. *Int J Cancer*. 2021;149(4):778-789.
4. Goldschmidt M, Peña L, Rasotto R, Zappulli V. Classification and grading of canine mammary tumours. *Vet Pathol*. 2011;48(1):117-131.
5. Han B, Li X, Yu Y. Role of β 2-microglobulin in cancer immunotherapy resistance. *Front Immunol*. 2025;16:11968357.
6. Huang WC, Wu D, Xie Z, Zhau HE, Nomura T, Zayzafoon M, *et al*. β 2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis. *Cancer Res*. 2006;66(18):9108-9116.
7. Jackson DC, Drayton IA, de Silva P, Frelinger JM, Robinson JJ, Croft SJ. Structure and function of β 2-microglobulin in MHC class I molecules. *Immunol Rev*. 1999;172:67-77.
8. Nosalova N, Takacova M, Bhide L. Canine mammary tumours: Classification, biomarkers and therapeutic approaches. *Int J Mol Sci*. 2024;25:2891.
9. Ploegh HL. Assembly and transport of MHC class I molecules. *Annu Rev Cell Biol*. 1988;4:229-253.
10. Prizment AE, Folsom AR, Cerhan JR, *et al*. Circulating β 2-microglobulin and risk of cancer. *Cancer Epidemiol Biomarkers Prev*. 2016;25(4):657-664.

11. Queiroga FL, Lopes CS. Tumour-associated antigens in canine mammary tumours: A review. *Vet J*. 2011;187(3):295-305.
12. Reis B, Duran J, Sanz M. Tumour β 2-microglobulin and HLA expression dynamics during immunotherapy. *Front Immunol*. 2024;15:1285049.
13. Sorenmo KU, Worley DR, Goldschmidt MH. Tumours of the mammary gland. In: Withrow SJ, Vail DM, editors. *Withrow and MacEwen's Small Animal Clinical Oncology*. 4th ed. St. Louis (MO): Saunders Elsevier; 2009. p. 619-636.
14. Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide. *CA Cancer J Clin*. 2021;71(3):209-249.
15. Zhang W, Wang H, Song SW, Fuller GN. Overexpression of β 2-microglobulin is associated with poor prognosis of gliomas. *J Neurooncol*. 2013;113(2):197-205.