(Appended form No. 3)

Abstract of Doctoral Thesis

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Title: Evaluation of Tumour-Infiltrating Immune Cells and Their Prognostic Impact in Feline Oral Squamous Cell Carcinoma and Appendicular Osteosarcoma
(猫の口腔扁平上皮癌および四肢骨肉腫における腫瘍浸潤免疫細胞の評価とその予後への影響)

Abstract of thesis:

Cancer is a major cause of morbidity and mortality in domestic animals, including cats. Among feline tumour, carcinomas are the most common diagnosed neoplasms, with oral squamous cell carcinoma representing the predominant subtype and accounting for approximately 60–70% of all oral tumours. This malignancy is typically aggressive, associated with a poor prognosis, and demonstrates limited responsiveness to conventional treatment modalities, particularly surgical excision. Although less frequent, osteosarcoma is the most common primary bone tumour in cats, comprising approximately 70% of primary bone neoplasms. In contrast to canine osteosarcoma, which is highly aggressive and prone to early metastasis, feline osteosarcoma generally exhibits slower biological behaviour and a comparatively lower metastatic rate, suggesting potential differences in tumour biology and host immune interactions between species.

Recent advances in cancer research have highlighted the importance role of the tumour microenvironment in influencing tumour initiation, progression, and therapeutic response. In particular, tumour-infiltrating immune cells have emerged as key regulators within this microenvironment, capable of exerting both antitumour and protumour effects depending on their phenotype, functional state, and spatial distribution. Immune cell populations such as T lymphocytes, B lymphocytes, and macrophages interact dynamically with tumour cells, shaping disease progression. In human oncology, increased infiltration of $CD3^+$ T cells, $CD8^+$ cytotoxic T cells, and $CD20^+$ B cells are frequently associated with favourable clinical outcomes across multiple tumour types, including squamous cell carcinoma and osteosarcoma. Conversely, tumour-associated macrophages are often linked to immunosuppression, tumour progression, and poorer survival outcomes. Despite these advances, the immunological landscape of feline cancers remains insufficiently characterised. In particular, the relationship between immune cell infiltration and clinicopathological parameters or survival outcomes in feline OSCC and osteosarcoma remains poorly understood, highlighting a critical gap in veterinary oncology. This study aimed to characterise tumour-infiltrating immune cells comprehensively and to evaluate their prognostic significance in feline OSCC and osteosarcoma.

A total of 36 feline tumour samples were retrospectively analysed, including 20 cases of OSCC and 16 cases of osteosarcoma. Formalin-fixed, paraffin-embedded tissue specimens were utilised, with OSCC samples collected between January 2008 and December 2022, and osteosarcoma samples collected between April 2007 and November 2012. Immunohistochemistry was performed to evaluate $CD3^+$ T cells,

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CD8$^+$ T cells, Foxp3$^+$ regulatory T cells, CD20$^+$ B cells, and CD204$^+$ macrophages. Associations between immune cell infiltration, clinicopathological features, and survival outcomes were assessed using Kaplan–Meier analysis and the log-rank test.

In feline oral squamous cell carcinoma, CD3$^+$ and CD8$^+$ T cells were widely distributed in both tumour and stromal regions, whereas CD20$^+$ B cells were predominantly localised in the stroma. CD204$^+$ macrophages and Foxp3$^+$ regulatory T cells showed variable distribution. Correlation analyses demonstrated significant associations among multiple immune cell populations, particularly within the stromal compartment, suggesting coordinated immune cell recruitment or shared regulatory mechanisms. Importantly, higher densities of stromal CD20$^+$ B cells and tumour-associated CD3$^+$ T cells were significantly associated with shorter progression-free intervals. Furthermore, increased tumour CD3$^+$ T cell infiltration was associated with reduced overall survival. Kaplan–Meier survival analysis revealed that lower infiltration of stromal CD8$^+$ T cells and tumour CD3$^+$ T cells was significantly associated with improved clinical outcomes. These findings suggest that, contrary to expectations based on human oncology, increased T cell infiltration in feline OSCC may not reflect an effective antitumour immune response.

In feline osteosarcoma, CD204$^+$ macrophages were the most abundant immune cell population, followed by CD3$^+$ and CD8$^+$ T cells, while CD20$^+$ B cells and Foxp3$^+$ regulatory T cells were less prominent. Significant correlations were observed among selected immune cell populations, particularly between CD3$^+$ and CD8$^+$ T cells, indicating potential functional interactions. Clinically, increased CD3$^+$ T cell density was significantly associated with poor prognosis, whereas CD8$^+$ T cells and macrophages demonstrated weaker or less consistent associations with survival outcomes. Additionally, a decrease in CD8$^+$ T cell density with advancing age was observed, suggesting a possible decline in immune surveillance in older cats. Overall, greater infiltration of CD3$^+$ and CD8$^+$ T cells was consistently associated with shorter survival, further supporting the notion that immune infiltration in feline tumours may not necessarily correlate with effective tumour control.

In conclusion, tumour-infiltrating immune cells are prognostically relevant in feline oral squamous cell carcinoma and osteosarcoma. Notably, increased infiltration of CD3$^+$ and CD8$^+$ T cells was associated with poorer survival, suggesting a potentially dysfunctional or immunosuppressive immune response rather than effective antitumour activity. These findings highlight the need for further investigation into immune regulatory mechanisms and support the development of prognostic markers and targeted immunotherapeutic strategies in feline cancers.

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（別紙様式第10号）

学位論文審査の結果の要旨

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題　　目	Evaluation of Tumour-Infiltrating Immune Cells and Their Prognostic Impact in Feline Oral Squamous Cell Carcinoma and Appendicular Osteosarcoma （猫の口腔扁平上皮癌および四肢骨肉腫における腫瘍浸潤免疫細胞の評価とその予後への影響）

審査結果の要旨：

猫の悪性腫瘍において、腫瘍微小環境を構成する免疫細胞は、腫瘍の進展や治療反応性に深く関与すると考えられている。しかし、猫の腫瘍における腫瘍浸潤免疫細胞の構成や予後との関係については知見が乏しい。本研究は、猫の代表的な悪性腫瘍である口腔扁平上皮癌および四肢骨肉腫を対象として、CD3陽性T細胞、CD8陽性T細胞、Foxp3陽性制御性T細胞、CD20陽性B細胞およびCD204陽性マクロファージを免疫組織化学的に評価し、臨床病理学的因子ならびに生存期間との関連を明らかにすることを目的として実施された。

第1章では、外科切除された猫の口腔扁平上皮癌20例を対象に、腫瘍間質および腫瘍実質内の各免疫細胞密度を定量した。CD3陽性T細胞およびCD8陽性T細胞は多くの症例で両領域に認められ、CD20陽性B細胞は腫瘍実質内より間質内に有意に多く分布していた。各免疫細胞の浸潤密度には相互に正の相関が認められ、免疫細胞が協調して集積する腫瘍微小環境の存在が示された。一方、免疫細胞密度と腫瘍ステージ、腫瘍径、リンパ節転移などの臨床病理学的因子との明確な関連は認められなかった。予後解析では、間質内CD20陽性B細胞および腫瘍実質内CD3陽性T細胞の高密度浸潤が短い無増悪期間と相関し、腫瘍実質内CD3陽性T細胞の増加は全生存期間の短縮とも関連した。また、Kaplan-Meier解析により、間質内CD8陽性T細胞および腫瘍実質内CD3陽性T細胞の浸潤が少ない症例で、より良好な予後が示された。これらの結果は、免疫細胞の多い腫瘍

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が必ずしも有効な抗腫瘍免疫を反映せず、免疫抑制やT細胞機能不全を伴う可能性を示している。

第二章においては、断脚術を受けた猫の四肢骨肉腫16例を対象に、腫瘍中心部の免疫細胞密度を後ろ向きに評価した。原発部位は大腿骨が最も多く、経過中に遠隔転移を認めた症例は43.8%であり、全生存期間中央値は320日であった。免疫組織化学的解析では、CD204陽性マクロファージが最も豊富で、次いでCD3陽性T細胞、CD8陽性T細胞、CD20陽性B細胞、Foxp3陽性制御性T細胞の順であった。CD3陽性T細胞、CD8陽性T細胞およびCD204陽性マクロファージの密度には正の相関が認められ、マクロファージに富む炎症性腫瘍微小環境が示唆された。高密度のCD3陽性T細胞浸潤は短い生存期間と有意に関連し、CD8陽性T細胞についても同様の傾向が認められた。また、CD3陽性およびCD8陽性T細胞密度は年齢と負の相関を示した。ただし、本研究は少数例を対象とする後ろ向きの探索的研究であり、免疫細胞の機能や空間的分布、免疫チェックポイント分子は評価していないことから、予後因子としての確立には前向き検証が必要である。

本学位論文は、猫の口腔扁平上皮癌および四肢骨肉腫における腫瘍浸潤免疫細胞を体系的に解析し、その一部が予後と関連することを明らかにした。一般に抗腫瘍免疫を担うと考えられるT細胞やB細胞の高密度浸潤が、予後不良と関連した点は重要であり、猫の腫瘍では免疫細胞数のみでは免疫応答の有効性を判断できないことを示している。これらの所見は、免疫抑制性マクロファージ、T細胞疲弊、免疫チェックポイント経路などの機能的解析へとつながる基盤的知見であり、猫の腫瘍における予後評価法および免疫療法の開発に寄与するものと考えられる。

本研究は、限られた猫腫瘍組織を用いながら、複数の免疫細胞マーカーを統一した方法で定量し、臨床情報および生存期間との関係を適切な統計手法により検討している。症例数、後ろ向き研究、単変量解析、機能的評価を欠くことなどの限界はあるものの、猫の腫瘍免疫微小環境に関する新たな知見を提示し、獣医腫瘍学および比較腫瘍学の発展に資する研究成果であると判断される。

以上により、本論文は博士（獣医学）の学位論文として十分な価値を有すると判断された。