

博士論文（要約）

イヌの膀胱癌における腫瘍浸潤リンパ球の役割と
その遊走メカニズムに関する研究

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緒論

本章の内容は、学術雑誌論文として出版する計画があるため公表できない。5年以内に出版予定。

第一章

イヌの膀胱癌における腫瘍浸潤リンパ球が予後に与える影響

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第二章

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制御性 T 細胞の浸潤抑制による抗腫瘍効果の検討

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總 括

本章の内容は、学術雑誌論文として出版する計画があるため公表できない。5年以内に出版予定。

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引用文献

1. V. Shankaran *et al.*, IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* **410**, 1107-1111 (2001).
2. W. H. Fridman, F. Pages, C. Sautes-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* **12**, 298-306 (2012).
3. G. P. Dunn, A. T. Bruce, H. Ikeda, L. J. Old, R. D. Schreiber, Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* **3**, 991-998 (2002).
4. F. C. Kurschus, D. E. Jenne, Delivery and therapeutic potential of human granzyme B. *Immunol Rev* **235**, 159-171 (2010).
5. P. Sharma *et al.*, CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci U S A* **104**, 3967-3972 (2007).
6. J. Galon *et al.*, Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**, 1960-1964 (2006).
7. L. Zhang *et al.*, Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* **348**, 203-213 (2003).
8. A. Tanaka, S. Sakaguchi, Regulatory T cells in cancer immunotherapy. *Cell Res* **27**, 109-118 (2017).
9. H. Nishikawa, S. Sakaguchi, Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol* **27**, 1-7 (2014).
10. D. M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* **12**, 252-264 (2012).
11. A. J. Mutsaers, W. R. Widmer, D. W. Knapp, Canine transitional cell carcinoma.

- J Vet Intern Med* **17**, 136-144 (2003).
12. D. W. Knapp *et al.*, Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. *ILAR J* **55**, 100-118 (2014).
 13. K. Saeki, A. Fujita, N. Fujita, T. Nakagawa, R. Nishimura, Total cystectomy and subsequent urinary diversion to the prepuce or vagina in dogs with transitional cell carcinoma of the trigone area: a report of 10 cases (2005-2011). *Can Vet J* **56**, 73-80 (2015).
 14. K. N. Khan, D. W. Knapp, D. B. Denicola, R. K. Harris, Expression of cyclooxygenase-2 in transitional cell carcinoma of the urinary bladder in dogs. *Am J Vet Res* **61**, 478-481 (2000).
 15. D. W. Knapp *et al.*, Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. *J Vet Intern Med* **8**, 273-278 (1994).
 16. D. W. Knapp, R. C. Richardson, G. D. Bottoms, R. Teclaw, T. C. Chan, Phase I trial of piroxicam in 62 dogs bearing naturally occurring tumors. *Cancer Chemother Pharmacol* **29**, 214-218 (1992).
 17. C. M. Fulkerson, D. W. Knapp, Management of transitional cell carcinoma of the urinary bladder in dogs: a review. *Vet J* **205**, 217-225 (2015).
 18. G. Redelman-Sidi, M. S. Glickman, B. H. Bochner, The mechanism of action of BCG therapy for bladder cancer--a current perspective. *Nat Rev Urol* **11**, 153-162 (2014).
 19. D. H. Aggen, C. G. Drake, Biomarkers for immunotherapy in bladder cancer: a moving target. *J Immunother Cancer* **5**, 94 (2017).
 20. N. N. Davarpanah, A. Yuno, J. B. Trepel, A. B. Apolo, Immunotherapy: a new treatment paradigm in bladder cancer. *Curr Opin Oncol*, (2017).

21. T. F. Gajewski, H. Schreiber, Y. X. Fu, Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* **14**, 1014-1022 (2013).
22. S. F. Ziegler, FOXP3: of mice and men. *Annu Rev Immunol* **24**, 209-226 (2006).
23. J. D. Fontenot *et al.*, Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* **22**, 329-341 (2005).
24. S. A. Rosenberg, N. P. Restifo, Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* **348**, 62-68 (2015).
25. C. M. O'Connor *et al.*, Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy. *Sci Rep* **2**, 249 (2012).
26. M. D. Robinson, D. J. McCarthy, G. K. Smyth, edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140 (2010).
27. X. Zhou, H. Lindsay, M. D. Robinson, Robustly detecting differential expression in RNA sequencing data using observation weights. *Nucleic Acids Res* **42**, e91 (2014).
28. M. D. Robinson, A. Oshlack, A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol* **11**, R25 (2010).
29. R. Yoshitake *et al.*, Molecular investigation of the direct anti-tumour effects of nonsteroidal anti-inflammatory drugs in a panel of canine cancer cell lines. *Vet J* **221**, 38-47 (2017).
30. T. J. Curiel *et al.*, Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* **10**, 942-949 (2004).
31. M. Gobert *et al.*, Regulatory T cells recruited through CCL22/CCR4 are

- selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res* **69**, 2000-2009 (2009).
32. D. Sugiyama *et al.*, Anti-CCR4 mAb selectively depletes effector-type FoxP3+CD4+ regulatory T cells, evoking antitumor immune responses in humans. *Proc Natl Acad Sci U S A* **110**, 17945-17950 (2013).
 33. R. Ueda, Clinical Application of Anti-CCR4 Monoclonal Antibody. *Oncology* **89 Suppl 1**, 16-21 (2015).
 34. M. C. Tan *et al.*, Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer. *J Immunol* **182**, 1746-1755 (2009).
 35. C. Y. Zhang *et al.*, The role of CCL20/CCR6 axis in recruiting Treg cells to tumor sites of NSCLC patients. *Biomed Pharmacother* **69**, 242-248 (2015).
 36. L. Ren *et al.*, Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer. *Oncotarget* **7**, 75763-75773 (2016).
 37. N. Redjimi *et al.*, CXCR3+ T regulatory cells selectively accumulate in human ovarian carcinomas to limit type I immunity. *Cancer Res* **72**, 4351-4360 (2012).
 38. S. Maeda *et al.*, Increase of CC chemokine receptor 4-positive cells in the peripheral CD4 cells in dogs with atopic dermatitis or experimentally sensitized to Japanese cedar pollen. *Clin Exp Allergy* **34**, 1467-1473 (2004).
 39. H. Mochizuki, K. Kennedy, S. G. Shapiro, M. Breen, BRAF Mutations in Canine Cancers. *PLoS One* **10**, e0129534 (2015).
 40. H. Mochizuki, S. G. Shapiro, M. Breen, Detection of BRAF Mutation in Urine DNA as a Molecular Diagnostic for Canine Urothelial and Prostatic Carcinoma. *PLoS One* **10**, e0144170 (2015).

41. T. Ishida *et al.*, Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. *J Clin Oncol* **30**, 837-842 (2012).
42. M. Tominaga *et al.*, Flow cytometric analysis of peripheral blood and tumor-infiltrating regulatory T cells in dogs with oral malignant melanoma. *J Vet Diagn Invest* **22**, 438-441 (2010).
43. M. I. Carvalho *et al.*, Intratumoral FoxP3 expression is associated with angiogenesis and prognosis in malignant canine mammary tumors. *Vet Immunol Immunopathol* **178**, 1-9 (2016).
44. J. H. Kim *et al.*, Correlation of Foxp3 positive regulatory T cells with prognostic factors in canine mammary carcinomas. *Vet J* **193**, 222-227 (2012).
45. B. J. Biller, A. Guth, J. H. Burton, S. W. Dow, Decreased ratio of CD8+ T cells to regulatory T cells associated with decreased survival in dogs with osteosarcoma. *J Vet Intern Med* **24**, 1118-1123 (2010).
46. J. H. Kim *et al.*, Infiltrating Foxp3+ regulatory T cells and histopathological features in canine classical and spermatocytic seminomas. *Reprod Domest Anim* **48**, 218-222 (2013).
47. A. Marcinowska *et al.*, T Lymphocytes in Histiocytic Sarcomas of Flat-Coated Retriever Dogs. *Vet Pathol* **54**, 605-610 (2017).
48. L. Mitchell, S. W. Dow, J. E. Slansky, B. J. Biller, Induction of remission results in spontaneous enhancement of anti-tumor cytotoxic T-lymphocyte activity in dogs with B cell lymphoma. *Vet Immunol Immunopathol* **145**, 597-603 (2012).
49. D. Pinheiro *et al.*, Dissecting the regulatory microenvironment of a large animal model of non-Hodgkin lymphoma: evidence of a negative prognostic impact of

- FOXP3+ T cells in canine B cell lymphoma. *PLoS One* **9**, e105027 (2014).
50. S. Maeda, K. Ohno, A. Fujiwara-Igarashi, K. Uchida, H. Tsujimoto, Changes in Foxp3-Positive Regulatory T Cell Number in the Intestine of Dogs With Idiopathic Inflammatory Bowel Disease and Intestinal Lymphoma. *Vet Pathol* **53**, 102-112 (2016).