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Identification of Suitable T-Cell Associated Transcripts for the Development of a New Veterinary Diagnostic Test for T-Cell Lymphoma

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Lymphoma is one of the most frequently encountered malignancies in veterinary practice, particularly in cats and dogs, but veterinary pathologists struggle with current diagnostic techniques to distinguish T-cell lymphomas from infiltrates of benign T-cells. Clonality studies are possible for cat and dog, as in human clinical pathology, but these are complex and time-consuming with variable success rates.

In human pathology, we have developed a new chromogenic in situ hybridisation (CISH)-based assay for formalin fixed paraffin embedded histological samples to look for T-cell monotypia, in a manner analogous to kappa/ lambda for B-cells. We determine the ratio of the TRBC1: TRBC2 constant segments in T-cell populations. Significant skewing away from the normal 1:1 ratio indicates likely lymphoma. In order to apply this approach to veterinary samples, we set out to identify the animal sequences, to determine their relative levels of expression and to provide preliminary CISH-staining data.

By aligning publicly available sequence data with the human TRBC1/2 sequences, the T-cell receptor constant regions, TRBC1 and TRBC2, were predicted for cat, dog and mouse. These were amplified from cDNA samples by PCR and confirmed by Sanger sequencing. Sequences from multiple animals showed greater polymorphism in cat and dog, with implications for CISH probe design. As for the human sequences, the 3' untranslated region shows the greatest variation between TRBC1 and TRBC2, making this a promising site for segment-specific CISH probe design. Q-PCR indicated a TRBC1: TRBC2 ratio close to 1:1 in cat, dog and mouse. TRBC1 and TRBC2 specific probes, produced by PCR and labelled with digoxygenin, gave excellent CISH staining for both TRBC1 and TRBC2 in lymphoid cells and on FFPE mouse spleen tissue.

In summary, we have demonstrated that CISH-based detection of TRBC1/2 could have utility as a test for animal T-cell lymphoma as it is likely to have for human T-cell lymphoma.