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Tumour-specific microRNA expression pattern in canine intestinal T-cell-lymphomas

Diana Joos[1] | Miriam Leipig-Rudolph[2,3] | Karin Weber[1]

[1]Clinic of Small Animal Medicine, Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Munich, Germany

[2]Specialty Practice for Veterinary Pathology von Bomhard and Pfleghaar, Munich, Germany

[3]Institute of Veterinary Pathology at the Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Munich, Germany

Correspondence
Dr Karin Weber, Clinic of Small Animal Medicine, Ludwig-Maximilians-Universität München, Veterinärstrasse 13, 80539 München, Germany.
Email: karin.weber@lmu.de

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Abstract

Intestinal T-cell lymphomas are common in dogs, but histopathological diagnosis remains challenging because of accompanying enteritis with lymphocyte involvement. Invasively taken full-layer biopsies are still required for reliable differentiation. The detection of specific microRNA expression patterns in canine intestinal T-cell lymphoma could provide new possibilities to differ intestinal lymphoma from benign inflammation and could lead to further understanding of lymphomagenesis. The objective of this study was to characterize microRNA expression in distinct groups of formalin-fixed and paraffin-embedded samples from canine intestinal T-cell lymphomas, lymphoplasmacellular enteritis and healthy intestinal tissue. In a preliminary test with two samples per group, total RNA was extracted (RNEasy FFPE Kit, Qiagen), reverse transcribed (miScript II RT Kit, Qiagen) and pre-amplified (miScript PreAmp PCR Kit, Qiagen). We performed comparative quantitative PCR on microRNA PCR Array plates (Qiagen) with pre-fabricated reactions for 183 different mature canine microRNAs. Subsequently, 12 microRNAs with conspicuous expression changes in the lymphoma group were selected and microRNA expression of all samples (n = 8) per group was analysed with individual microRNA assays (miScript Primer Assays, Qiagen) on the reverse transcribed RNA without pre-amplification. Our results revealed lymphoma-specific expression patterns, with down-regulation of the tumour-suppressing microRNAs miR-194, miR-192, miR-141 and miR-203, and up-regulation of oncogenic microRNAs, including microRNAs from the miR-106a~363 cluster. In addition, we detected only slight expression alterations between healthy intestinal tissue and lymphoplasmacellular enteritis cases. We conclude that microRNA expression patterns can be used to separate T-cell lymphomas from healthy tissue and benign inflammatory disorders.

KEYWORDS

dog, lymphoma, microRNA, reverse transcriptase polymerase chain reaction

1 | INTRODUCTION

Malignant lymphoma is one of the most frequent canine malignancies, with an increasing age-adjusted occurrence up to 107 cases per 100 000 dogs annually.[1] Following multi-centric lymphoma, gastrointestinal lymphoma represents the second commonest lymphoma form, accounting for approximately 5 to 7% of all canine lymphoma cases.[2] The most commonly affected anatomic location is the small intestine,

and neoplastic cells show pre-dominantly T-cell origin.[3,4] In comparison to the multi-centric form, the prognosis of intestinal lymphoma is poor, with median survival times of 13 to 77 days despite multi-agent chemotherapy and/or surgical intervention.[5,6] The definite diagnosis of canine intestinal lymphoma remains challenging, since the neoplastic lymphomatous lesions are often masked by concomitant benign inflammatory reactions in the mucosa, especially lymphoplasmacellular enteritis (LE).[2] To achieve accurate results, a stepwise diagnostic approach using full-thickness biopsies beginning with morphological examination, followed by immunophenotyping and determining the Ki67 index and finally PCR for antigen receptor gene rearrangement analysis (PARR) to characterize clonality, is recommended.[7]

MicroRNAs (miRNAs) are a class of small (~22 nucleotides), single-stranded, non-coding RNA molecules found in almost all eukaryotic cells, which post-transcriptionally regulate protein synthesis by base pairing to partially complementary sequences in the 3′ untranslated regions (UTRs) of target mRNAs.[8-10] MiRNAs influence a variety of cell signalling pathways and can act either as oncogenes or as tumour suppressors.[11,12] More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites.[13]

In humans, miRNA expression in B-cell lymphomas has been studied extensively, but less is known about miRNA expression in T-cell lymphomas.[14,15] In a study analysing human cutaneous T-cell lymphomas, miRNA expression patterns differed significantly from both benign inflammatory skin diseases and normal skin. A diagnostic accuracy of over 95% in the differentiation benign/malignant was achieved using a set of only three differentially expressed miRNAs (miR-155, miR-203, miR-205).[16] The progression of an early stage of mycosis fungoides to advanced cutaneous T-cell lymphoma appears to be accompanied by the overexpression of several oncogenic miRNA clusters, indicating miRNA profiling might be helpful for both diagnosis and risk prediction in cancer.[17] Overexpression of the miR-106a~363 cluster in human T-cell lymphomas was described in 2007, and the oncogenic potential was proven in a transgenic mouse model, where forced overexpression induced T-cell lymphoma.[18,19]

Few studies have investigated miRNA expression patterns in canine lymphoma. In non-neoplastic lymph nodes and in lymph nodes of lymphoma cases, the expression of a small miRNA-panel was analysed and compared. The expression patterns of B- and T-cell lymphomas were different from normal tissue and from each other.[20] In primary canine splenic lymphoma, the miR-17-5p/miR-155 M ratio was investigated as a potential new marker for grading.[21] Another study investigated the miRNA expression in lymph nodes of lymphoma cases and canine B- and T-cell lymphoma cell lines and revealed an increased expression of oncogenic miRNAs and down-regulation of known tumour-suppressing miRNAs compared with non-neoplastic lymph nodes.[22] A recent study determined the profile of circulating miRNAs in serum samples from dogs with and without lymphoma. The sample set included dogs with several different anatomic forms of lymphoma, and the immunophenotype (B-cell or T-cell lymphoma) was not assessed. Several miRNAs were found to be differentially expressed in the lymphoma group compared with the healthy group.[23] To the best of our knowledge, no studies on miRNA expression in canine intestinal T-cell lymphoma have been reported to date.

The purpose of our study was to characterize miRNA expression in well-defined groups of FFPE samples from canine intestinal T-cell lymphomas, lymphoplasmacellular enteritis and healthy intestinal tissue. Using canine-specific miRNA-arrays and assays, we could detect lymphoma-specific expression patterns, with marked down-regulation of tumour-suppressing miRNAs and up-regulation of oncogenic miRNAs, including miRNAs from the miR-106a~363 cluster.

2 | MATERIALS AND METHODS

2.1 | Ethic statement

The study was approved by the XXX Committee on Research Ethics (Number: 58-23-10-2015).

2.2 | Sample collection

Formalin-fixed, paraffin-embedded full-thickness tissue specimens of canine small intestine that had been taken for diagnostic purposes were used for this study. All samples were analysed by histopathological examination by certified veterinary pathologists. The samples were classified in three groups (n = 8 each): healthy control group (C), lymphoplasmacellular enteritis (LE) and intestinal T-cell lymphoma (T) (Table 1). The final diagnosis T-cell lymphoma had been confirmed in an earlier study via a combination of histopathological examination, immunohistochemistry using CD3, CD20 and CD79a antibody staining and polymerase chain reaction for antigen receptor gene rearrangement analysis (PARR).[4] Two tumour samples showed large nuclei and high-grade mitosis, two samples showed large nuclei and intermediate grade mitosis, one sample showed large nuclei and low-grade mitosis and three samples showed intermediate nucleus size and high-grade mitosis. Two cases of LE were also confirmed by immunohistochemistry staining and PARR, the remaining LE cases were diagnosed by histopathological examination only.

2.3 | MicroRNA expression analysis

2.3.1 | RNA extraction

Total RNA including microRNA was isolated from two to four 10 μm thick tissue sections using the miRNEasy FFPE Kit (QIAGEN, Hilden, Germany). The sections were treated with Deparaffinization Solution (QIAGEN), and RNA was extracted according to the manufacturer's instructions. Total RNA was eluted with 30 μL of RNase-free water. RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware).

TABLE 1 Details of the patient groups

	T-cell lymphoma (n = 8)
Age	Median, 8.1 years; range, 5-11 years
Sex	Male, 5; female, 3 (spayed 1)
Breed	6 pure breeds (Golden Retriever, Bernese Mountain Dog, Poodle, Yorkshire Terrier, Belgian Shepherd, Maltese); 1 mixed breed; 1 not specified
	Lymphoplasmacellular enteritis (n = 8)
Age	Median, 7.1 years; range, 1–12 years
Sex	Male, 4 (castrated 2); female, 4 (spayed 3)
Breed	4 pure breeds (Yorkshire Terrier, Magyar Viszla, Labrador Retriever, Coton de Tulear); 4 mixed breeds
	Healthy control (n = 8)
Age	Median, 7.7 years; range, 6 weeks-12 years
Sex	Male, 3; female, 5 (spayed 3)
Breed	7 pure breeds (Dachshund, Yorkshire Terrier, Golden Retriever, Entlebucher Mountain Dog, Austrian Black and Tan Hound, German Shepherd, White Shepherd); 1 not specified

Note: Characteristics of the dogs in the T-cell lymphoma group (n = 8), lymphoplasmacellular enteritis group (n = 8) and healthy control group (n = 8) used in the study.

2.3.2 | Expression analysis using microRNA arrays

As a preliminary test, microRNA expression was analysed in two samples of each group on custom-made microRNA arrays (384-well plate, QIAGEN) containing 183 different mature canine microRNAs, four expression controls (SNORD 61, SNORD 68, SNORD 95 and RNU6-6P), one reverse transcription control and one PCR control for each sample. Total RNA (100 ng) was reverse transcribed using the miScript II RT Kit (QIAGEN). The cDNA was pre-amplified using the miScript PreAmp PCR Kit (QIAGEN) according to the manufacturer's instructions on a Mastercycler (eppendorf AG, Hamburg, Germany) with a miScript PreAmp universal primer and a custom miScript PreAmp primer mix. The pre-amplified cDNA was diluted with 100 µL RNase-free water.

The array plates were run on a 7900 HT Sequence Detection System (Applied Biosystems, ABI PRISM, Foster City, California) using the miScript SYBR Green PCR Kit (QIAGEN) with a reaction volume of 10 µL per well. The amplification protocol consisted of an initial activation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds and extension at 70°C for 30 seconds. In order to account for spurious PCR amplification of contaminating genomic DNA, a control containing total RNA without reverse transcription was included. The plates were inspected by melting curve analysis after amplification to confirm a single PCR product in each well. Relative quantification was carried out using the miScript miRNA PCR Array Data Analysis software (Qiagen) using three different normalization methods (normalization with SNORD95 and RNU6-6P, normalization using the global Ct mean of all expressed miRNAs, and normalization using four miRNAs cfa-miR-631/cfa-miR-23b/cfa-miR-320/cfa-miR-125a automatically selected by the software).

2.3.3 | Expression analysis using microRNA assays

Based on the expression results of the microRNA arrays, 12 canine microRNA assays and two microRNA control assays were selected, and microRNA expression was analysed on all samples of the C, LE and T group (n = 8 each). RNA extraction was carried out as described for the arrays.

Reverse transcription was performed using the miScript II RT Kit (QIAGEN) according to the manufacturer's instructions with 1700 ng total RNA per sample. The cDNA was diluted 1:25 with RNase-free water, and the samples were analysed by real-time PCR without pre-amplification.

Real-time PCR was run on a QuantStudio 6 Flex System (Applied Biosystems) using the miScript SYBR Green PCR Kit (QIAGEN) with an universal primer (QIAGEN) and specific miScript Primer Assays (QIAGEN). The assays hs_RNU_6-2 and hs_SNORD95 were used as endogenous control assays. As target assays, cf_miR-194, cf_miR-18b, cf_miR-203, cf_miR-141, cf_miR-20b, cf_miR-192, cf_miR-210, cf_miR-363, cf_miR-34a, cf_miR-9, cf_miR-142, cf_miR-130b and cf_miR-19a were selected. All samples were run as duplicates in a reaction volume of 10 µL, containing 5 µL QuantiTect SYBR Green PCR Master Mix, 1 µL miScript Universal Primer, 1 µL miScript Primer Assay, 2 µL RNase-free water and 1 µL of the diluted RT reaction. The amplification protocol was run as described for the arrays. In order to account for spurious PCR amplification of contaminating genomic DNA, a control containing total RNA without reverse transcription was included. The plates were inspected by melting curve analysis after amplification to confirm a single PCR product in each well. Relative quantification was carried out using the miScript miRNA PCR Array Data Analysis software (Qiagen) with SNORD95 and RNU6 2 as endogenous controls.

3 | RESULTS

3.1 | MiRNA expression profiling using MiRNA Arrays

Expression of miRNAs was measured in two samples per group (Healthy intestinal tissue, T-cell lymphoma and lymphoplasmacellular enteritis) using custom-made miRNA arrays. From the 183 mature canine miRNAs tested, quality control showed that few (0.5-3%) miRNAs were not expressed or below threshold, 3% to 22% were expressed with Ct values between 25% and 30%, and 75% and 91% were expressed with Ct values < 25. The expression results of the arrays are shown in Supplementary Table S1. A large number of miRNAs (> 50%) were differentially expressed more than 2-fold between control tissue and both the lymphoma and enteritis group. For further analysis, 12 miRNAs were selected using the criteria overall expression (Ct values < 25), high differential expression between groups, and available information on possible functional roles in lymphoma pathophysiology.

Table 2 shows the expression differences of 12 selected miRNAs on the arrays when using different normalization strategies. While the

TABLE 2 Expression differences in the arrays T-cell lymphoma vs healthy using different normalization methods

miRNA	RNU6B/ SNORD95	Global Ct mean	Automatically selected
cfa-miR-194	−81.0	−44.0	−19.4
cfa-miR-192	−101.5	−55.1	−24.3
cfa-miR-141	−52.9	−28.8	−12.7
cfa-miR-203	−42.3	−23.0	−11.1
cfa-miR-19a	1.3	2.4	5.5
cfa-miR-34a	−2.5	−1.4	1.68
cfa-miR-142	5.7	10.5	23.9
cfa-miR-130b	3.7	6.8	15.4
cfa-miR-9	2.7	5.0	11.4
cfa-mir-363	22.3	41.9	93.3
cfa-miR-20b	10.6	19.6	44.4
cfa-miR-18b	41.2	75.8	172.3

Note: Three different normalization methods were applied to the miRNA expression arrays. The fold regulation of 12 selected miRNAs is shown for a comparison T-cell lymphoma vs healthy control for normalization with RNU6B and SNORD95, for normalization using the Global Ct mean of all expressed miRNAs, and for normalization using four miRNAs automatically selected by the software.

expression changes differ in absolute numbers, the trends for up- or down-regulation are concordant regardless of the normalization method.

3.2 | MiRNA expression profiling using miRNA assays

Expression of the 12 selected miRNAs was tested on all samples (n = 8 per group) with individual miRNA assays. The expression changes between healthy intestinal tissue and lymphoplasmacellular enteritis cases were not statistically significant when tested with individual assays (all *P*-values > .05). The expression changes between normal intestinal tissue and T-cell lymphoma cases (down- or up-regulation) could be confirmed and were statistically significant in 11 miRNAs. The expression changes between lymphoplasmacellular enteritis cases and tumour tissue were similar to the comparison healthy intestinal tissue vs tumour tissue and statistically significant in six miRNA assays (Table 3). Hierarchical cluster analysis using the expression results of the 12 selected miRNAs separated a group of 7 T-cell lymphoma cases from the other samples, while samples of healthy intestinal tissue and lymphoplasmacellular enteritis could not be discriminated by this method (Figure 1).

4 | DISCUSSION

This study provides the first overview of miRNA expression patterns of FFPE samples from intestinal T-cell lymphoma, lymphoplasmacellular enteritis and healthy enteric tissue in dogs. The initial screening with arrays for canine miRNA revealed high amounts of detectable miRNAs in canine enteric tissue and differences in miRNA expression levels between healthy control tissue, tissue from dogs with lymphoplasmacellular enteritis, and neoplastic tissue. Since different normalization methods can affect the expression results considerably, we used three normalization methods on the miRNA arrays. For the selected miRNAs, the results were concordant for up- and down-regulation regardless of the normalization method. This finding is in agreement with a study on miRNA expression in samples from colorectal cancer, which showed that highly differentially expressed miRNAs can be detected using different reference genes or combinations of reference genes.[24]

TABLE 3 miRNA expression analysis

	T-cell lymphoma vs healthy			Enteritis vs healthy			T-cell lymphoma vs enteritis		
miRNA	Array	Assay	*P* value assay	Array	Assay	*P* value assay	Array	Assay	*P* value assay
cfa-miR-194	−81.0	−95.7	.0006	−5.9	−1.3	.7009	−19.9	−72.6	.0045
cfa-miR-192	−101.5	−64.1	.0012	−4.9	1.1	.2834	−28.9	−72.2	.0039
cfa-miR-141	−52.9	−56.8	.0002	−4.3	−2.5	.3861	−17.1	−22.9	.0270
cfa-miR-203	−42.3	−19.0	.0001	−3.8	−1.1	.4599	−15.7	−17.1	.0008
cfa-miR-19a	1.3	−4.9	.0009	6.7	−2.5	.4004	−7.2	−2.0	.0941
cfa-miR-34a	−2.5	−2.5	.0383	−8.1	−1.3	.9907	2.4	−1.9	.1660
cfa-miR-142	5.7	3.1	.0008	−1.1	1.8	.0822	4.6	1.7	.0622
cfa-miR-130b	3.7	4.4	.0057	−2.5	1.4	.1524	6.5	3.2	.0163
cfa-miR-9	2.7	6.7	.1118	−8.9	1.1	.3063	17.3	5.9	.1319
cfa-mir-363	22.3	9.0	.0003	−1.6	1.8	.1155	24.9	4.9	.0016
cfa-miR-20b	10.6	10.3	.0002	−2.9	2.0	.1374	22.1	5.1	.0032
cfa-miR-18b	41.2	13.6	.0002	1.4	4.6	.1476	21.0	2.9	.7950

Note: Fold regulation of 12 selected miRNAs in T-cell lymphoma compared with healthy intestinal tissue, lymphoplasmacellular enteritis compared with healthy intestinal tissue and T-cell lymphoma compared with lymphoplasmacellular enteritis using miRNA arrays (n = 2 samples per group) and miRNA assays (n = 8 samples per group). *P*-values < .05 were considered significant.

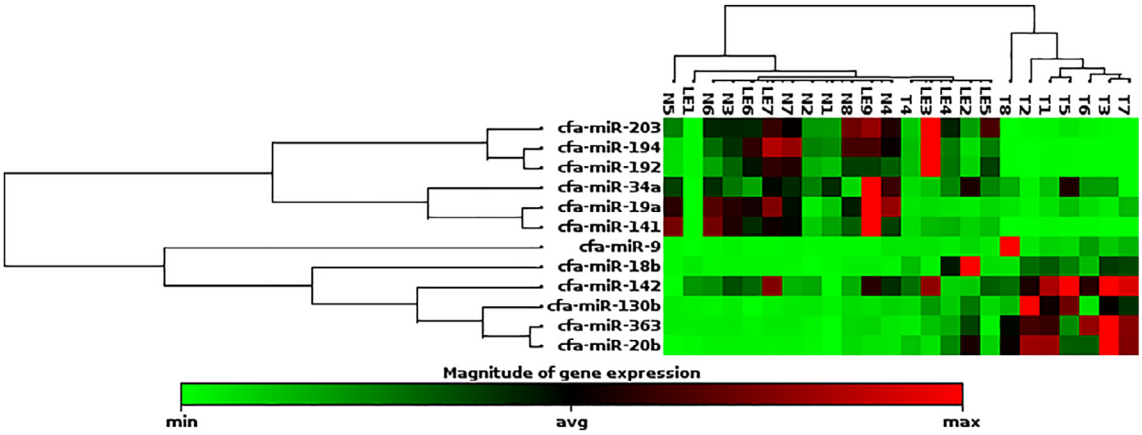

FIGURE 1 Cluster analysis. The colour intensity from *green* to *red* shows the degree of down-regulation (*green*) to up-regulation (*red*) compared with the other samples. A group of seven T-cell lymphoma cases (T) clusters on the right side, while nearly all samples of the lymphoplasmacellular enteritis group (LE) as well as the healthy control group (N) cluster on the left side. MiRNAs with tumour suppressor function like miR-194, miR-192, miR-141 and miR-203 show low expression levels in T-cell lymphoma samples, whereas miRNA-18b, miR-20b and miR-363, which belong to the oncogenic cluster miR-106a~363, are strongly expressed in the tumour group

In a subsequent test with larger sample groups, the expression of several individual miRNAs was found to be significantly different in a group of canine intestinal T-cell lymphomas. In comparison to the other two groups, up-regulation of the miRNAs miR-18b, miR-20b and miR-363 combined with decreased expression of miR-194, miR-192, miR-141 and miR-203 was observed.

So far, there is only limited knowledge about miRNA expression patterns in canine lymphoma and existing studies mainly focused on their diagnostic applicability. In 2010, the expression of a small group of 11 miRNAs was quantified and compared between non-neoplastic lymph nodes and lymphoma samples from FFPE and fresh-frozen tissue.[20] The results of the fresh-frozen samples were consistent with those of the FFPE samples, which suggests that our findings obtained from archive material are transferable to freshly obtained samples. In the lymph node samples of canine T-cell lymphomas, miR-181a was significantly elevated and miR-29b significantly decreased compared with healthy lymph nodes.[20] In our study, both miRNAs showed no expression differences in any of the groups. Possible explanations for discrepancies among several expression analyses are biological variations between lymphoma sub-types, the usage of different assays (human MicroRNA Taqman assay or SYBR Green based assay) contributing to experimental bias, or the selected type of normalization.[22,25,26]

Another miRNA expression analysis in lymphoma diseased lymph nodes (B- and T-cell lymphomas) of dogs detected increased amounts of oncogenic miRNAs and down-regulation of tumour-suppressing miRNAs compared with peripheral blood mononuclear cells (PBMC) and healthy lymph nodes. In comparison to healthy lymph nodes, the miRNAs miR-106a, miR-17-5p and miR-20a showed an augmented expression in canine T-cell lymphoma samples.[22] For miR-106a, we found a slightly increased expression in our lymphoma samples, and a distinctively higher expression of the miRNAs miR-18b, miR-20b and miR-363. The miRNAs miR-106a, miR-18b, miR-20b and miR-363 are encoded within the miR-106a~363 polycistronic cluster, consisting of 6 adjacent miRNAs located on chromosome X in mice and humans.[27] Prooncogenic effects have been reported for the miR-106a~363 cluster and its two paralogons miR-17~92 and miR-106b~25 in a variety of tumours, indicating their critical role in carcinogenesis.[27] Correspondingly, miR-106a~363 overexpression was detected in 46% of human T-cell leukemias, comparing blood and marrow samples from acute and chronic T-leukaemia cases to healthy peripheral blood mononuclear cells (PBMC).[19] A further study compared FFPE samples from healthy lymph nodes with samples from human anaplastic large cell lymphomas, an aggressive T-cell lymphoma, revealing high expression levels of miR-20b and miR-106a.[28] Moreover forced expression of the miR-106a~363 cluster during thymocyte maturation led to the formation of severe T-cell lymphomas in transgenically modified mice.[18] Furthermore, small RNA sequencing of bone marrow from human T-cell acute lymphoblastic leukaemia patients compared with healthy controls, revealed overexpression of three miRNAs of the miR-106a~363 cluster, for which an oncogenic effect by inhibition of apoptotic gene targets was demonstrated.[29] Taken together, our findings of miR-106a~363 deregulation in FFPE samples from canine intestinal T-cell lymphomas agree with similar investigations in humans and mice, which highlights the possibility for cross-species regulatory pathways of miRNAs in T-cell lymphomas.

For miR-194 and miR-192, which belong to the related miRNA clusters miR-194-2~miR-192 and miR-194-1~miR-215, down-regulation and convergent tumour suppressive effects in human malignancies such as colorectal cancer, renal cell carcinoma and multiple myeloma have been reported.[30-32] In addition, lower miR-194 and miR-192 expression levels in peripheral blood or bone marrow samples of human acute lymphoblastic leukaemia, compared with healthy controls, were described,[33] and a tumour suppressive function through cell cycle interference was successfully demonstrated for miR-192 in an acute lymphoblastic leukaemia cell line.[34] Likewise, decreased miR-192 levels in marrow samples from patients with acute myeloid leukaemia compared with healthy controls, as well as miR-192 dependent tumour suppressive cell cycle regulation in two acute

myeloid leukaemia cell lines have been observed.[35] In line with these findings, our analysis also showed miR-192 and miR-194 down-regulation in the malignant tissue compared with the other two groups. Although miR-215 was not tested with a singleplex assay, it shows a very low expression compared with the two other groups in the arrays. This is in line with the down-regulation of miR-192 and miR-194, since these three miRNAs were found to have common targets and form a network of tumour suppressors.[31,36,37] To the best of our knowledge, studies on the functional role of these miRNAs in dogs have not been published to date.

The miR-141 down-regulation found in the tumour group in our study shows parallels to an investigation in humans, which revealed significantly decreased miR-141 expression levels in cutaneous T-cell lymphomas compared with inflammatory skin diseases via microarray profiling in skin biopsies.[38] A tumour suppressive function was demonstrated for miR-141 in hepatocellular carcinoma cells, preventing tumour cell invasion and migration by targeting the T lymphoma invasion and metastasis (Tiam1) gene.[39]

A tumour suppressive function has been shown for miR-203 in human haematopoietic malignancies, where a loss of its expression leads to up-regulation of the oncogene ABL-1.[40] MiR-203 inactivation may be because of both genetic and epigenetic mechanisms and epigenetic silencing more frequently occurs in lymphoid than myeloid malignancies.[40,41] Similar to our findings, a significantly decreased miR-203 expression in FFPE samples from human cutaneous T-cell lymphomas compared with benign skin disorders and healthy skin, was reported. Besides miR-203, miR-205 levels were also considerably decreased in the neoplastic samples compared with benign diseases.[16] However, miR-205 showed no conspicuous expression changes between the different groups in our study.

The significance of our results was limited because of the small number of samples and the use of archive material, although the results obtained from FFPE samples and freshly frozen tissue should match well.[20] Since we did not excise the tumour region from the slides for analysis the miRNA expression represents a mixture of tissues, which might dilute tumour specific changes. Inflammatory processes in both enteritis and tumour samples might lead to concordant shifts in some miRNAs. Tumour sample T4 shows a miRNA expression pattern that is quite different to the other tumour samples. The histological pattern of this tumour shows a low mitose rate, but large nuclei. Information about any pre-treatment or treatment of the animals and of the aggressiveness and clinical behaviour of the tumours is lacking, which adds to the variability of the results, and the reason for the different expression pattern remains elusive. Since we focused on highly differentially expressed miRNAs, miRNAs with medium and small differences were not analysed, but could also have important biological effects in this tumour entity.

Taken together, our study revealed a distinct miRNA expression pattern in canine intestinal T-cell lymphoma. In particular, the increased expression of the oncogenic miRNAs miR-18b, miR-20b and miR-363 and down-regulation of the tumour suppressive miRNAs miR-192, miR-194, miR-141 and miR-203 was noted. The spontaneously developing tumours of the dog may serve as an additional model for the role of miRNAs in tumour pathophysiology and as diagnostic biomarkers, or can even be used to test the effectiveness of miRNA-based tumour therapeutics, which may benefit both dogs and humans.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Karin Weber https://orcid.org/0000-0002-0541-4355

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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