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MIRNA PROFILES IN DOGS WITH SPLENIC ANGIOSARCOMA

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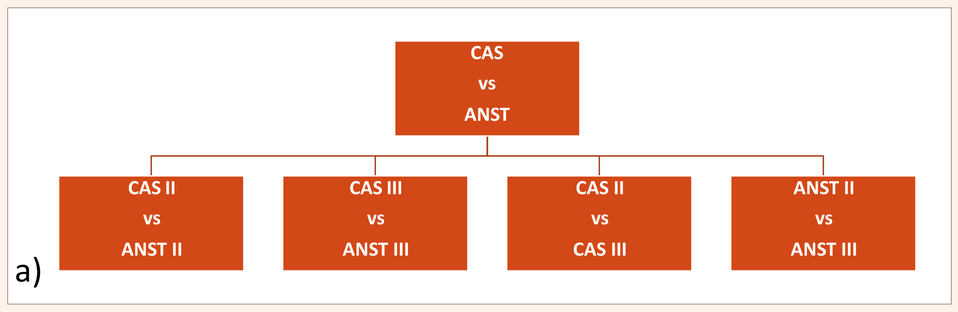
Introduction and Aims

MiRNAs are small single-stranded non-coding RNA molecules involved in mRNA silencing and post-transcriptional regulation of gene expression by binding to target mRNAs[1]. They have been demonstrated to be significantly dysregulated in neoplastic transformation, functioning as cancer promoters or suppressors[2].
AIMS were to assess miRNA profiles in formalin-fixed, paraffin-embedded canine splenic angiosarcomas (CAS) by next-generation sequencing

Materials & Methods

Twenty-four samples were included: 12 CAS of which 6 clinical stage II and 6 clinical stage III and 12 samples corresponding autologous normal splenic tissues (ANST) (Tab.1). Briefly, miRNAs were extracted from FFPE tissue punch biopsies using an miRNeasy FFPE kit. Small RNA transcripts were converted into bar-coded cDNA libraries. Library preparation was performed using an NEBNext Multiplex Small RNA Library Prep Set for Illumina, and sequencing was performed in a NextSeq 500 sequencer (methods' flowchart in Fig.1).



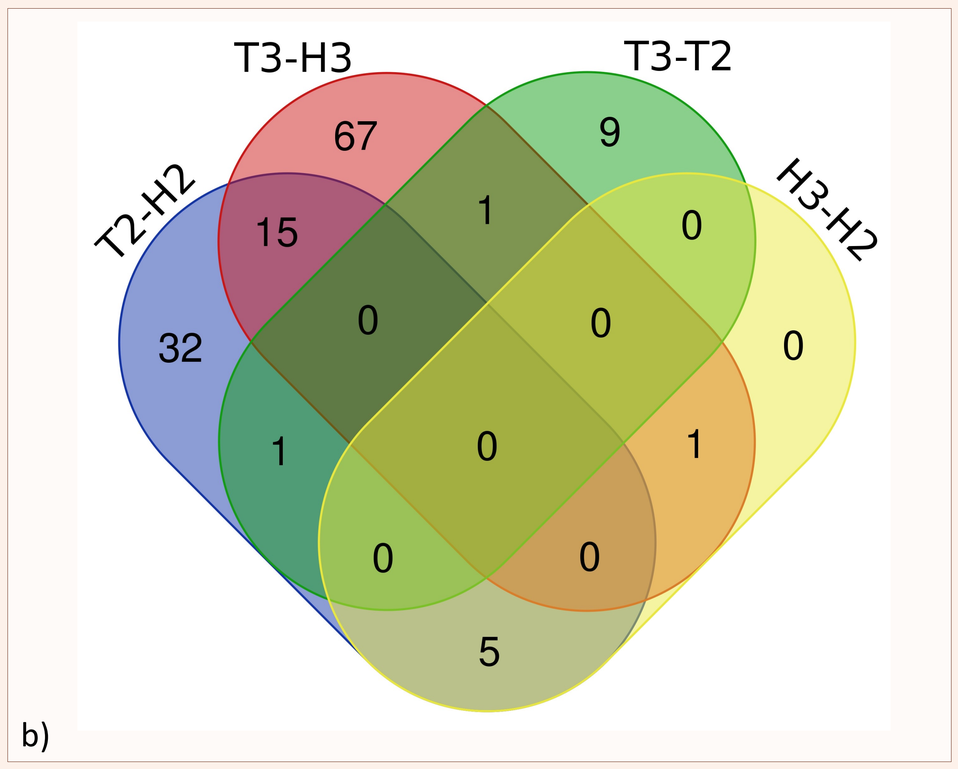


Fig.2. a) Diagram illustrating subdivision of cases into different groups; b) Venn diagram showing the relationship of the DE-miRNAs between the 4 groups. Legend: T2-T3 = Tumor stage 2, 3 (CAS II, III); H2-H3 = Healthy tissue (ANST II, III).

miRNA extraction from FFPE → **Library preparation** → **Sequencing** → **Data analysis**

Fig.1: Flowchart of the methods.

Results

The expression of 20 miRNAs was significantly differentially expressed (DE) in CAS compared with ANST.
Dividing CAS by clinical stage (Fig.2) and comparing the results with corresponding ANST, expression of DE-miRNAs resulted as follows:

- **53 DE-miRNAs in CAS vs ANST stage II;**
- **84 DE-miRNAs in CAS vs ANST stage III;**
- **11 DE-miRNAs (8 up- and 3 downregulated) in stage II vs stage III CAS;**
- **6 upregulated miRNAs in stage II vs stage III ANST (Tab.1).**

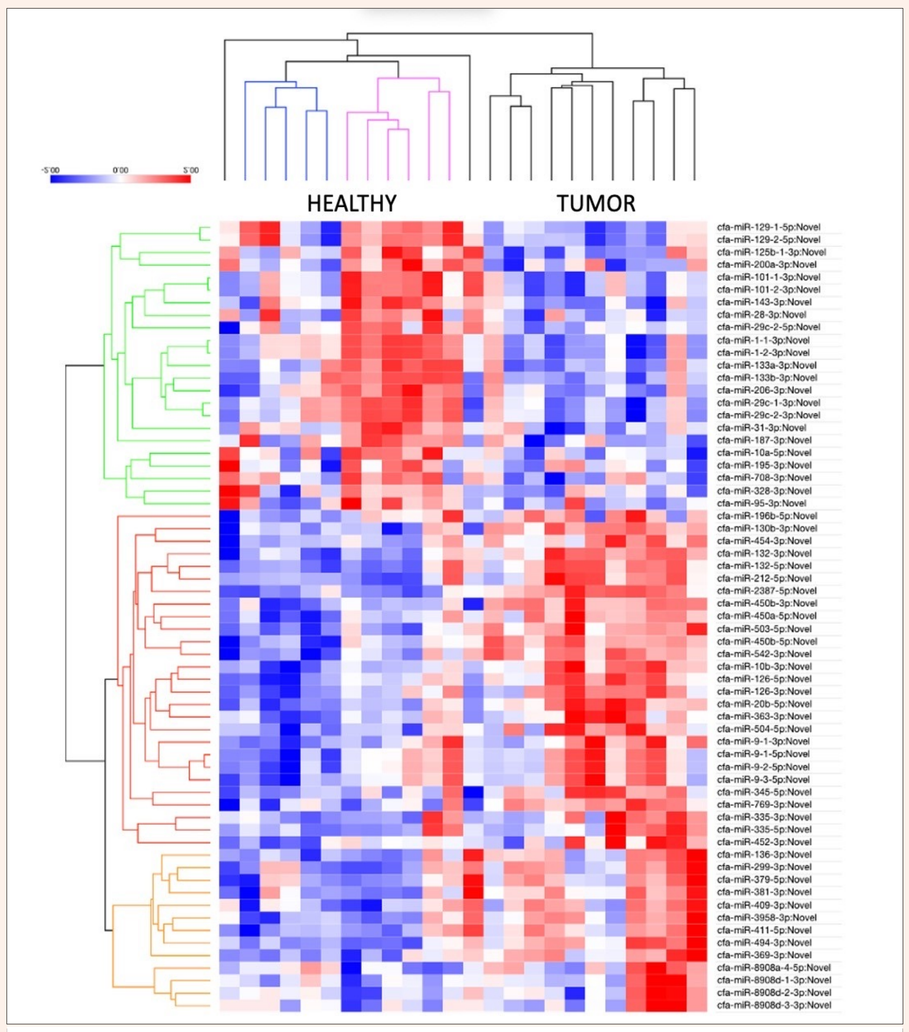


Fig.1. HeatMap of the DE-miRNAs in tumoral (CAS) independently of clinical stage and healthy tissues (ANST). The upper right part of the graph: dichothomy in ANST compared to CAS is evident.
Legend: Blue = low levels of miRNA, **Red** = high levels of miRNA.

Groups		De-miRNAs		Statistics	
Group 1	Group 2	Up regulated	Down regulated	pad j	log2FC
CAS	ANST	15	5	< 0,05	I1.4I
CAS II	ANST II	27	26	< 0,09	I1I
CAS III	ANST III	34	50		
CAS II	CAS III	8	3		
ANST II	ANST III	6	-		

Tab.1: DEmiRNAs (up- and down-regulated) evaluated between groups.

Discussion & Conclusions

This preliminary study suggests involvement of specific miRNAs in the epigenetic regulation in CAS. Furthermore, there is a difference between miRNA profile in stage II and stage III CAS and in ANST in dogs with stage II and stage III too, suggesting a possible tissue predisposition for neoplastic angiosarcoma development. Future analyses, including additional cases of CAS and including splenic tissues from dogs without CAS, are warranted to confirm these results.

References

(1) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–297
(2) Bracken CP, Scott HS, Goodall GJ. A network-biology perspective of microRNA function and dysfunction in cancer. Nat Rev Genet. 2016;17(12):719–732.