#2404

Change in Expression of MicroRNAs Following Limp Amputation in Canine Osteosarcoma Patients Predicts Survival Time After Chemotherapy

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Background

- Osteosarcoma (OSA) is the most common primary bone tumour in humans and dogs, presenting most often in the appendicular skeleton[1]
  - Dogs are a highly relevant, spontaneously metastasizing animal model for aggressive human OSA due to the higher prevalence and generally more aggressive nature of canine OSA compared to the human disease[1]
- Median survival following amputation and chemotherapy in dogs is only 1 year and 90% of dogs develop metastases following treatment[2]
  - Predicting outcome is difficult; only the presence of metastatic disease at initial diagnosis is predictive of post treatment survival time
- MicroRNAs (miRNAs) are small, non-coding RNA that regulate gene expression[3]
  - MiRNAs are good potential biomarkers for prognosis as they are readily measured in blood and are often dysregulated in cancer[3]
  - Tumour cells can secrete miRNAs into the blood, but after removing the primary tumour miRNA expression profiles are expected to change, which could be exploited to better prognosticate patients
  - MiRNAs also have completely, or nearly identical sequences in dogs and humans which supports cross-species utility[4]

Objectives

- Investigate prognostic significance of individual miRNAs using the change in expression between pre- and post-amputation plasma samples
- Build multi-miRNA decision trees using the change in expression between pre- and post-amputation plasma samples as an easy to use, prognostic test

Materials and Methods

Data

Samples

- 25 matched pre- and post-amputation plasma
- All dogs received standard of care treatment

MiRNA Expression

- 47 miRNAs quantified by reverse transcription real time PCR as cycle threshold (Ct) values

Survival Metrics

- Overall Survival: Time from diagnosis to death from osteosarcoma metastasis
- Progression Free Survival (PFS): Time from diagnosis to first evidence of metastasis or death
- Samples were censored at their last known timepoint if they died from non-OS related reasons, were still alive, or were lost to follow up

Data Processing

- Normalization
  - The NormFinder algorithm was used to select the top 3 stably expressed miRNAs to serve as endogenous controls[5]
  - Ct values were normalized using the ΔΔCt method → For each sample, the geometric mean of the 3 endogenous controls was subtracted from each miRNA of interest[6]
- Difference in Expression
  - For each matched pair of samples, the post-amputation Ct value was subtracted from the pre-amputation sample to calculate changes in expression (ΔCt values)

Materials and Methods [Continued]

Correlations Between miRNA Expression Change and Outcome

- Dogs were split into 2 groups using their ΔCt values based on the most significant cutoff as determined by surv_cutpoint() in the survminer package in R[7]
- Kaplan-Meier curves were used to visualize survival differences between the groups
  - p-values calculated using the Log-Rank Test
- The process was repeated for each individual miRNA in both overall survival and PFS metrics

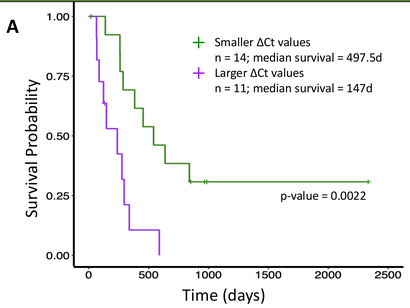
Multi-miRNA Survival Decision Trees

- Decision trees grown using rpart package in R[8]
  - Minsplit parameter set to 15 to pre-prune the tree
- Dogs were allocated to groups based on their final node in the tree
- Kaplan-Meier curves were used to visualize survival differences between groups
  - p-values calculated using Log-Rank Test
- Trees were grown for both overall survival and PFS metrics

Results

miRNA Expression Change and Outcome

Overall Survival



Progression Free Survival

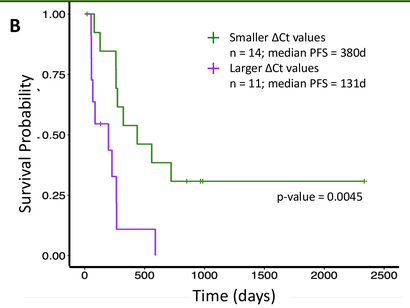


Figure 1: Kaplan-Meier Curves for miRNA-214 for overall survival (A) and PFS (B).
For both metrics, ΔCt values larger than the optimal cutoff of 1.71 (purple), indicating samples with larger reductions in expression following amputation, correlated with shorter overall survival and a shorter time until disease progression

Multi-miRNA Survival Decision Trees

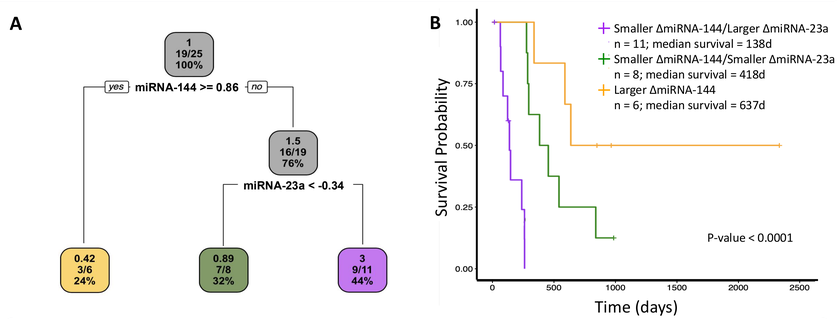


Figure 2: A multi-miRNA tree (A) for overall survival and corresponding Kaplan-Meier curves (B)
Elevated post-amp miRNA-144 and reduced post-amp miRNA-23a (purple) had the worse prognosis, elevated post-amp miRNA-144 and elevated miRNA-23a (green) had moderate prognosis, and a reduced post-amp miRNA-144 (yellow) had improved prognosis

Results [Continued]

Multi-miRNA Survival Decision Trees

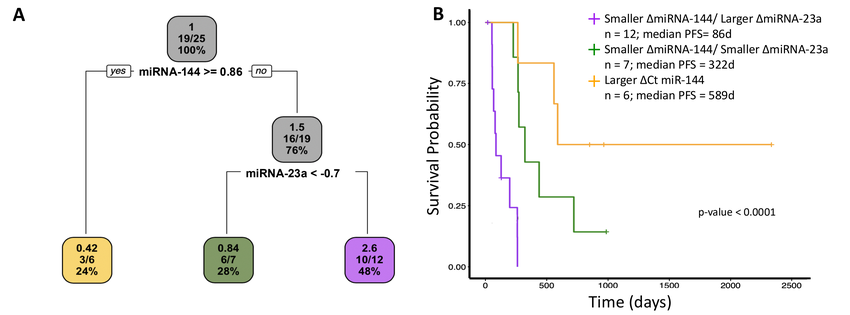


Figure 3: A multi-miRNA tree (A) for PFS and corresponding Kaplan-Meier curves (B)
Elevated post-amp miRNA-144 and reduced post-amp miRNA-23a (purple) had the shortest time to progression, elevated post-amp miRNA-144 and elevated miRNA-23a (green) had moderate time to progression, and reduced post-amp miRNA-144 (yellow) had the longest time to progression

Conclusions and Future Work

- Differences in the change in miRNA expression for single miRNAs following amputation can significantly discriminate between short and long survivors for both overall and PFS
- Multi-miRNA models are better able to differentiate patients with more “extreme” survival times from those with more typical, median prognoses for both overall survival and PFS
- Results reflect chemotherapy-independent changes in miRNA expression which could be used to better understand the biology of the primary OSA tumours
- These analyses should be tested on a larger, confirmatory population to ensure the models have generalized predictive power
- These results should be compared to the performance of pre- and post-amputation miRNA expression as independent predictors of prognosis to determine if changes in expression perform better

Acknowledgements

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OVC PET TRUST

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