Development of a Canine Appendicular Osteosarcoma *in vitro* Metastasis Assay Pipeline

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Introduction

Approximately 90% of dogs diagnosed with appendicular osteosarcoma will be euthanized within two years despite aggressive treatment.

Metastasis is a complex multi-step process where cells must migrate and invade the adjacent tissue, enter the vasculature, survive transport within the vasculature, arrest within a vessel, and extravasate to reach their secondary site where they form a colony.

Functional assays mimicking the steps of the metastatic cascade *in vitro* can help us understand the intricacies of metastatic disease and identify potential opportunities for therapeutic intervention.

Hypothesis: Certain osteosarcoma cell lines will be capable of performing specific steps of the metastatic cascade more effectively in *in vitro* metastasis assays.

Objectives: To establish an *in vitro* metastasis assay pipeline to investigate mechanisms of metastasis and to characterize the metastatic capacity of a diverse panel of canine osteosarcoma cell lines.

Canine Cell Line Panel

Primary Osteosarcoma Cell Lines: OVC-cOSA-31*, OVC-cOSA-75, OVC-cOSA-78, OVC-cOSA-106, Abrams, Basil, Duke, Dharma, Tonka

Commercial Osteosarcoma Cell Lines: D17*

Mesenchymal Canine Cell Lines: OVC-cOSA-103, OVC-cOSA-109

*metastatic origin

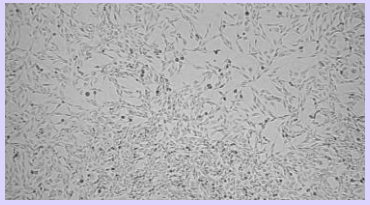

Fig 1. D17 canine osteosarcoma cell line in standard culture conditions exhibits adherent spindle-shaped morphology.

Materials and Methods

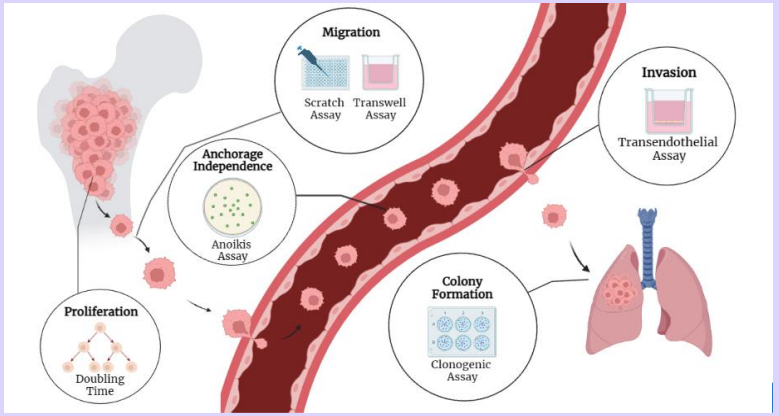

Fig 2. *in vitro* metastasis assay pipeline schematic demonstrating assays and their corresponding step of the metastatic cascade. Each assay will be performed using 3 wells per cell line and each assay will be repeated twice to determine the baseline metastatic abilities of 10 canine osteosarcoma and 2 normal mesenchymal cell lines.

Preliminary Results

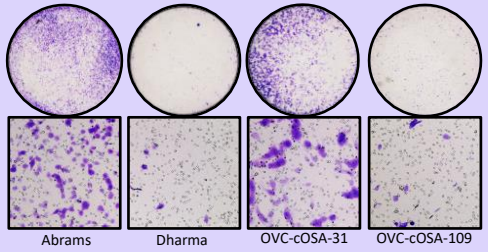

Fig 3. The transwell assay demonstrates the varying ability of serum-starved canine osteosarcoma and normal mesenchymal cells to migrate through a porous membrane towards serum-supplemented media.

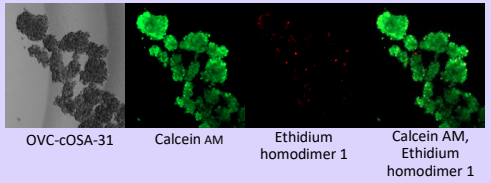

Fig 4. Canine osteosarcoma cell line OVC-cOSA-31 is capable of anchorage independent survival, as demonstrated in an anoikis assay through quantification of live (green) and dead (red) cells.

Results

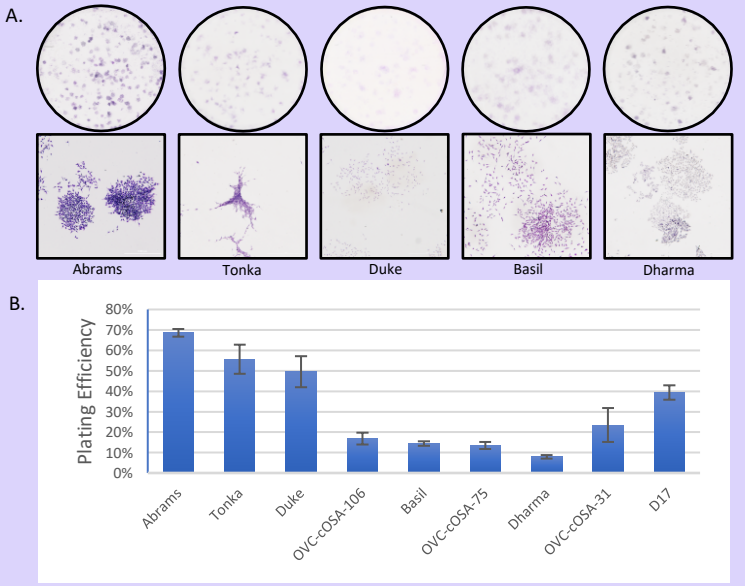

Fig 5. The clonogenic assay demonstrates the varying ability of canine osteosarcoma cells to form colonies from a single cell. **(A)** Colonies of varying morphology at low (top) and high (bottom) magnification. **(B)** Plating efficiency determined by quantifying the number of >50 cells colonies.

Discussion

Thus far, cell lines from this panel demonstrate variable capacity to migrate and form colonies. Assay optimization and cell line characterization is ongoing. Performance within these assays will elucidate which cell lines are best to investigate specific steps of the metastatic cascade and the pipeline will be invaluable for future drug studies and mechanistic investigations.

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