Evaluation of Canine Mast Cell Tumor Sensitivity to Oncolytic Viruses

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Background

- Canine cutaneous and subcutaneous mast cell tumors [MCTs] arise from malignantly transformed mast cells (MCs).
- Cancer is dogs' most common cause of natural death and while there are several standard treatments available for pet cancer, many of them have limited effectiveness. In human cancer therapy, there is a growing interest in oncolytic virotherapy.
- Oncolytic viruses (OVs) destroy tumor cells in two ways: (1) by directly infecting, replicating, and ultimately bursting cancer cells, and (2) by releasing antigens when the tumor cells die, which stimulate an immune response that fights against the tumor.
- This study evaluated different OVs for their therapeutic potential against a dermal canine MCT isolated from a 7-year-old male castrated Sharpei dog.

Methods

Cell Culture:

- To conduct cell infectivity and viability assays using flow cytometry and resazurin assays, MCT-1 cells were seeded at 8,300 cells/well in 96-well cell culture plates and allowed to adhere for 24 hours.

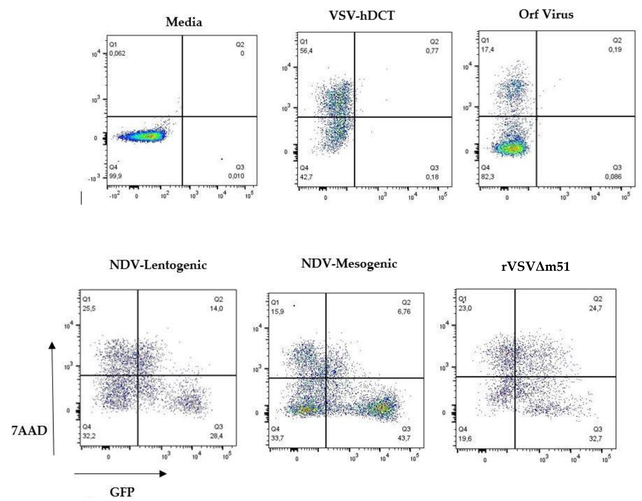
Using Flow Cytometry to Analyze Infections of OVs that Were Tagged With Green Fluorescent Protein (GFP):

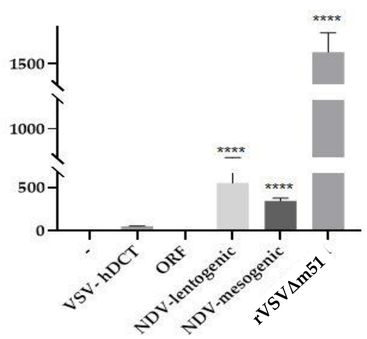
- MCT-1 cells were treated with GFP-tagged OVs rVSVΔm51, NDV, and ORFV at multiplicities of infection (MOI) 10. After 48 hours of incubation, a 7-aminoactinomycin D (7AAD) viability marker (Bio Legend; San Diego, CA, USA) was added to the wells and incubated at 4.0°C for 20 minutes. The cells were washed and run on a FACS-Canto II flow cytometer (BD; San Jose, CA). Live cells (7-AAD negative) were monitored for the expression of GFP.

Using Resazurin Assay to Analyse Cell Viability

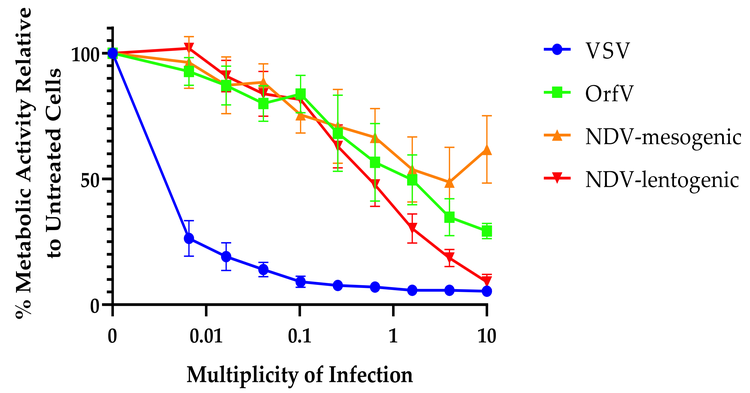
- Resazurin assays were conducted to quantify the metabolic activity of MCTs, which correlates with cell viability.
- The MCT-1 line was infected with VSV, Orf Virus, and mesogenic and lentogenic NDV. The cells were incubated for 48 hours. After 48 hours, 0.25 mg/mL resazurin sodium salt (Sigma-Aldrich, Oakville, ON, Canada) was added to the culture, and fluorescence was measured four hours later with a plate reader (excitation wavelength: 535/25 nm, emission wavelength: 590/35 nm).

Results





- **Destructive effect of OVs on MCT-1 shown by the number of cells that expressed GFP**. Cells were detected GFP-positive post-infection using flow cytometry. Dot plots are representative of GFP positive MCT-1 48h post-infection. Total number of GFP positive cells at 48h (Statistical significance as p ****<0.0001).



Differential Viral Oncolytic Efficacy in MCT-1. The cytotoxicity of rVSVΔm51, OrfV, NDV-mesogenic, and NDV-lentogenic on MCT-1 was assayed using metabolic resazurin assays. Statistical analysis was per-formed using a two-way ANOVA with Dunnett's multiple comparisons. P Values for onco-lytic activities were shown (b) (n=3; **** p<0.0001).

Conclusions & Future Directions

- Incorporating GFP in the OVs facilitates i) *in vitro* virus infection and ii) cell susceptibility monitoring.
- After incubation with the OVs, MCT-1 displayed GFP positivity, indicating that it is sensitive to OVs.
- To evaluate the efficacy of OVs against MCTs, other canine MCT cell lines will be utilized.
- Once all the necessary requirements have been met, we will proceed to enroll the findings in a clinical study.

Acknowledgements

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