Investigation of the PD-1/ PDL-1 pathway in histiocytic sarcoma of Flatcoated Retrievers

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INTRODUCTION

PD-L1 and PD-1 together form an immune checkpoint pathway:

- programmed cell death protein 1 (PD-1) and PD-L1 are cell surface glycoproteins
- PD-L1 expressed on 'antigen presenting cells'
- PD-L1 expression increased on many tumours, tumour-infiltrating lymphocytes and tumour-associated macrophages
- PD-1 expressed on activated T cells and highly expressed on regulatory T cells
- Interaction of PD-L1 with PD-1:

o promotes self-tolerance by promoting apoptosis in antigen specific T cells
o limits the activity of T cells during inflammation
o regulates the development, maintenance and function of regulatory T cells

Aims of the study
To investigate the expression of PD-L1 in canine histiocytic sarcoma by immunostaining and mRNA expression.

METHODS

Samples
Localised histiocytic sarcoma (HS) from flatcoated retrievers: archived frozen and FFPE blocks

Immunofluorescence
Frozen tumour samples and positive control: human intestine (not shown) stained with anti-PD-L1: rabbit anti-human CD274 (Novus) [1:100]. Negative control: canine tumour tissue stained with rabbit IgG.

RT-PCR
PD-L1 and PD-1 primers were designed and tested on RNA from FFPE blocks from spleen and 4 different tumours to confirm expression of mRNA.

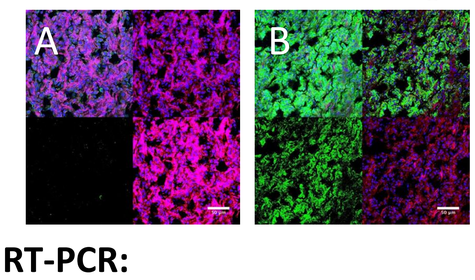
IMMUNOHISTOCHEMISTRY:
FFPE tumour samples and Positive control: canine spleen (not shown) stained with anti-PD-L1: rabbit anti-human CD274 (Novus) [1:150]. Negative control: canine spleen stained with rabbit IgG.

Canine histiocytic sarcoma:

- aggressive tumour
- cells of myeloid lineage
- flatcoated retrievers predisposed
- primary location:
  deep in musculature or in soft tissue surrounding joint
- can also arise in abdomianl organs (often spleen) «visceral form»
- prognosis always poor

RESULTS

IMMUNOFLUORESCENCE:
All 4 tumours tested showed positivity for PD-L1 on neoplastic cells, different levels of signal were observed amongst tumours.

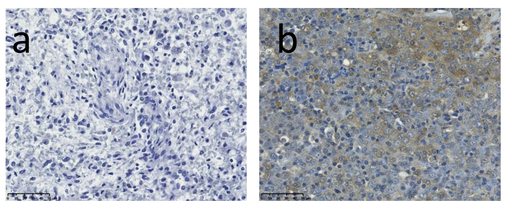


A. Isotype control
B. Anti-PD-L1 signal

RT-PCR:
All 4 tumour analysed with PD-L1 assay showed presence of mRNA, however quantitation was not achieved.

IMMUNOHISTOCHEMISTRY:
26 tumours were selected for IHC and all showed strong staining for PD-L1. On average 40% to 60% of neoplastic cells showed positive immunolabelling

a. Isotype control
b. Anti-PD-L1 staining



DISCUSSION

- This project has identified a possible role for the PD-1/PD-L1 pathway in canine HS immune evasion. All tumour samples asessed showed positive staining for PD-L1 with an average of 41-60% of neoplastic cells immunolabelled.
- The efficacy of treatments targeting the PD-1/PD-L1 immune check-point rely on the consequent increased T cell-specific immune response, allowing activation of the immune system to fight the tumour.
- There has been a recent patent application for a canine chimeric monoclonal antibody targeting PD-L1 and its efficacy has been tested in a clinical trial on malignant tumours.
- Clinical trials in dogs bearing HS might be a possibility in the near future.

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