Investigating the role of PD1-PDL1 tumour-intrinsic signalling in pathogenesis of canine oral melanoma

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Immune checkpoints are proteins that modulate organism's response to immunogenic stimuli. They are essential for maintaining self-tolerance, but cancer cells can exploit them to evade destruction by the host. A potent inhibitory checkpoint mediated by Programmed cell death protein 1 (PD1) consists of PD1 receptor -expressed on T and B lymphocytes - and ligands - PDL1 and PDL2 – on a variety of cells.

Melanoma continues to be a deadly cancer in humans and dogs, with high degree of disease similarity. PDL1 expression on cancer cells halts T-cell responses. PD1 receptor blockade with monoclonal antibodies significantly increases survival of human patients with advanced melanoma and other cancers. Interestingly, melanomas also contain cell populations expressing PD1 receptor, where PD1-PDL1 signaling activates mTOR pathway and enhances tumorigenicity, also in absence of a competent immune system. Blockade of tumour-intrinsic PD1-PDL1 axis may also contribute to the treatment efficacy.

I investigate the role of PD1-PDL1 tumour-cell intrinsic signaling in pathogenesis and progression of canine melanoma. As a disease model, canine tumours occur spontaneously and share many pathogenic pathways with the human ones. Dogs share the human owner's lifestyle and environmental exposures. Dogs and humans also have higher similarity in genetic code in comparison to mice.

Using flow cytometry, immunofluorescence and Western Blotting (WB) we have detected, that PD1 is expressed at a low level in all canine melanoma cell lines (CML10, CMGD2, CMGD5 and TLM1), and at a high level in histiocytic sarcoma cell line (DH82), which was used as positive control.

To characterize the PD1 and PDL1 expression in stem cell fraction, we have established melanoma spheroid cultures and evaluated transcripts and proteins levels by qPCR and WB respectively.

To identify the PD1 role in melanoma pathogenesis, we created PD1 and PDL1 overexpressing and CRISPR-based knock out cell lines, observed cellular phenotype, growth rate, invasiveness and stem-like characteristics. We performed PD1 blocking experiments using mAb developed in the project previously. We are currently optimizing production of recombinant anti-canine-PD1 antibody, testing

various methods of modulating PD1 and PDL1 expression, and assessing the impact of pro-inflammatory cytokines on PD1 and PDL1 proteins expression in cancer cells.