Canine oral melanoma: An investigation into the molecular bases of malignancy and refractoriness

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Background:

- Melanoma/malignant melanoma: malignant neoplastic proliferation of melanocytes.
- Melanomas represent approximately 40% of the malignant tumours of the oral cavity in the canine species.
- They possess a high metastatic potential.
- Neoplastic transformation of a normal cell is based on survival after genetic changes, avoiding the mechanism of apoptosis.
- Avoiding apoptosis is an important step in the metastatic dissemination.

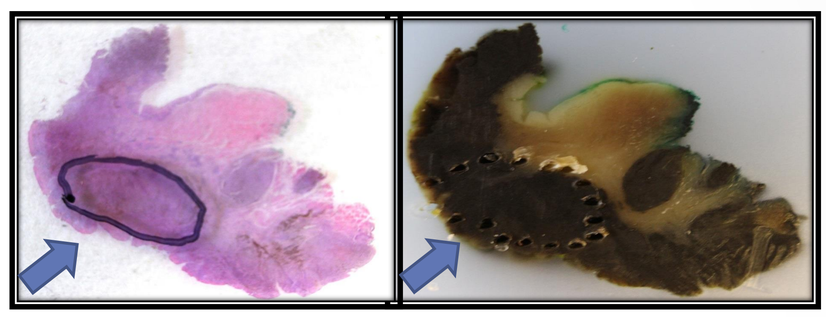
Aims:

- **Study A:** Investigation into the molecular bases of the high metastatic potential of canine oral melanoma using formalin-fixed paraffin-embedded (FFPE) samples.
- **Study B:** Investigation of the use of immunohistochemical staining for lymphatic endothelial cells as a tool to increase sensitivity of detection of lymphatic invasion in canine oral melanomas.

Study A:

Actual project status:

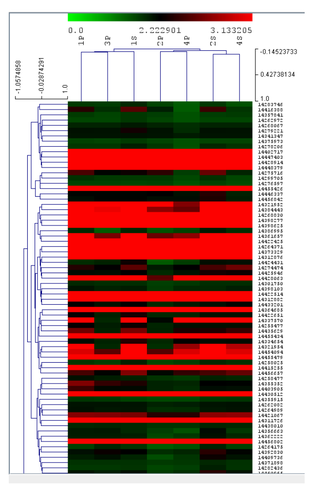
- Collected 11 cases of formalin-fixed, paraffin-embedded (FFPE) biopsies of primary oral melanoma and relative regional lymph nodal (LN) metastases in the database of the Pathology Department (Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK) and the database of the Departement Pathobiologie - Veterinair Pathologisch Diagnostisch Centrum (Univeristy of Utrecht) and isolated representing areas of the FFPE material.



Identification and isolation of representative area of the tumour on paraffin block

- Identified 5 potential cases from other external collaborators (Idexx Italy, Via Guglielmo Silva, 36, 20149 Milano, Italy and Idexx UK, Grange House, Sandbeck Way, Wetherby LS22 7DN).

- Extraction of RNA and DNA from 10 primary oral melanomas and matched LN metastasis.
- Evaluation of presence of adequate amount of RNA and DNA in the selected material (NanoDrop Nucleic Acid Quantification, Quant-iT™ RiboGreen® RNA Assay, Quant-iT™ PicoGreen™ dsDNA Assay).
- Evaluation of differences in gene expression between primary oral melanoma and relative regional lymph nodal metastasis by microarray mRNA profiling (4 pairs of primary tumour and LN metastasis)



Identification of 158 differentially expressed transcript clusters

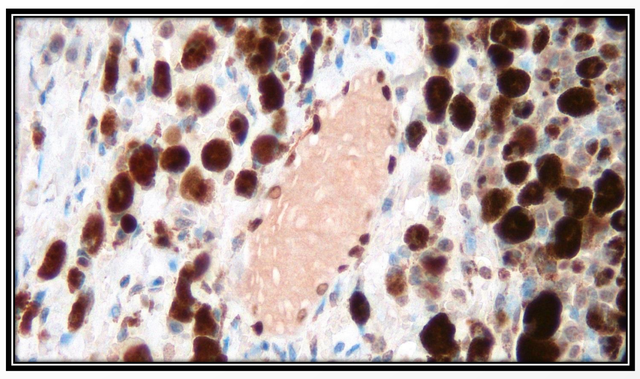
Next steps:

- Evaluation of correlation between the expression of different genes, focusing on correlation between the expressions of genes that share a particular functional annotation (which could be associated with metastasis) in this subset of primary oral melanomas and matched metastasis.
- Include all the other collected samples and potential newly recruited cases in the analysis.

Study B:

Actual project status:

- Identification of 10 primary oral melanomas from dogs with confirmed metastasis at diagnosis, and 10 oral melanomas from cases with confirmed lack of metastatic spread to the regional lymph node at diagnosis.
- Optimization of the immunohistochemical protocol for the lymphatic-specific marker PROX1 (rabbit anti-human, polyclonal, AngioBio, Del Mar, CA, USA) using FFPE canine tissue.



Positive PROX1 nuclear signal in the central vessel with melanoma cell in the adjacent tissue

Next steps:

- Perform immunohistochemistry for PROX1 of all the collected samples and recruit other cases (target is 15 metastatic and 15 non-metastatic melanomas).
- Investigate if IHC detection of lymphatic endothelial cells offers a greater sensitivity than H&E staining for detecting vascular invasion of melanoma cells in primary canine oral melanomas with confirmed lymph node metastasis.
- Evaluate if endothelial cell IHC-detected lymphatic invasion is associated with dissemination to the regional lymph node