

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

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.
- Melanomas possess a high metastatic potential.
- Lymphatic invasion by neoplastic melanocytes is associated with sentinel lymph node metastasis, and survival in melanoma cases.

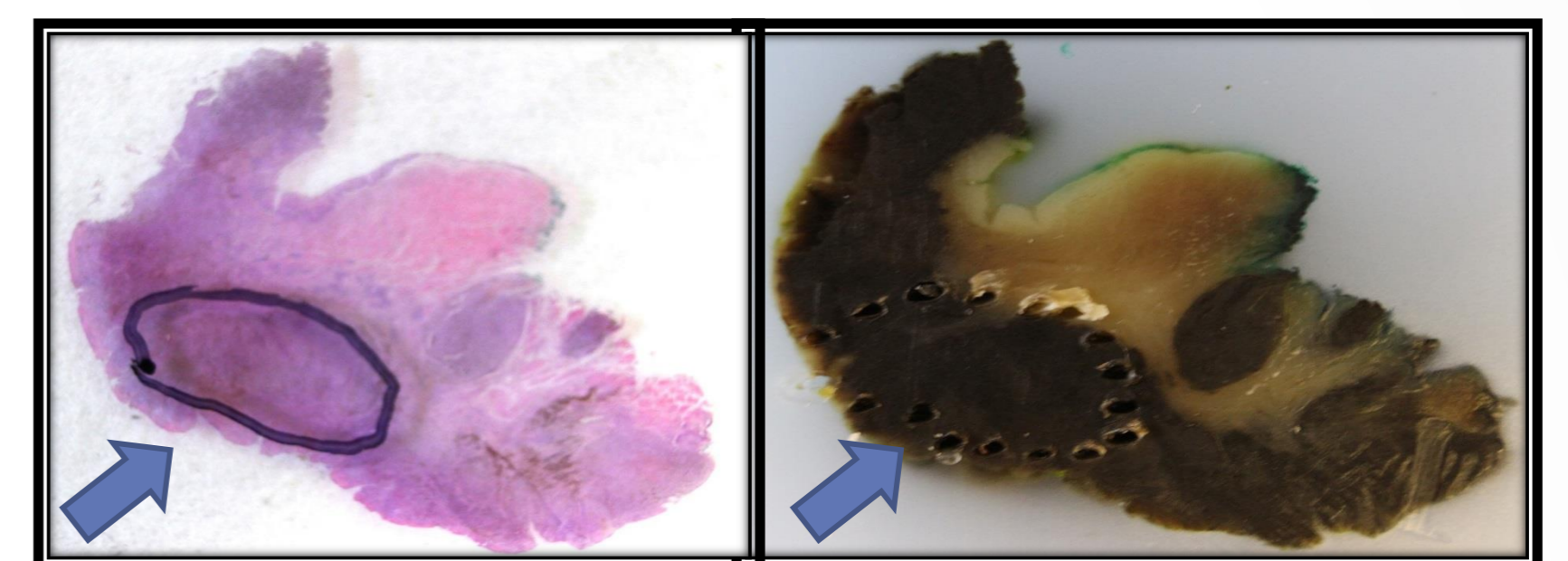
Aims:

- **Study A:** Investigation into the molecular bases of the high metastatic potential of canine oral melanoma by global molecular analyses of formalin-fixed paraffin-embedded (FFPE) primary and secondary melanomas.
- **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.

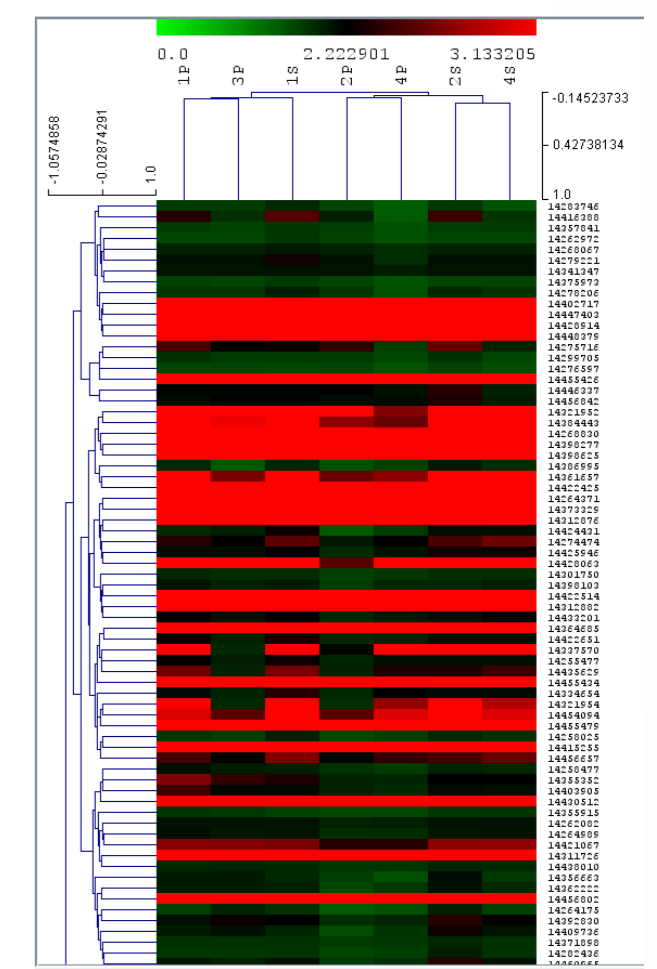
Study A:

Project status:

- Dissected melanocytes from FFPE biopsies of primary oral melanoma and relative regional lymph nodal (LN) metastases from 15 cases documented in the databases of the Pathology Department (Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK) and the Departement Pathobiologie – Veterinair Pathologisch Diagnostisch Centrum (University of Utrecht).
- Extracted RNA and DNA from 10 primary oral melanomas and matched LN metastasis.
- Quantified RNA and DNA (NanoDrop Nucleic Acid Quantification, Quant-iT RiboGreen RNA Assay, Quant-iT PicoGreen dsDNA Assay).
- Profiled gene expression in 4 primary tumours and LN metastases (Canine Gene 1.1 ST Arrays), and performed differential expression analysis.



Identification and isolation of a representative area of a melanoma in a paraffin block



Hierarchical clustering of primary melanomas and LN metastases

Results:

- 158 genes displayed differential expression (permutation testing-adjusted p-value <0.05, fold-change ≥ 2).
- Enriched gene functional annotations include 'cell adhesion molecules' and 'chemokine signaling pathway'
- Several genes involved in Rac-1 pathway

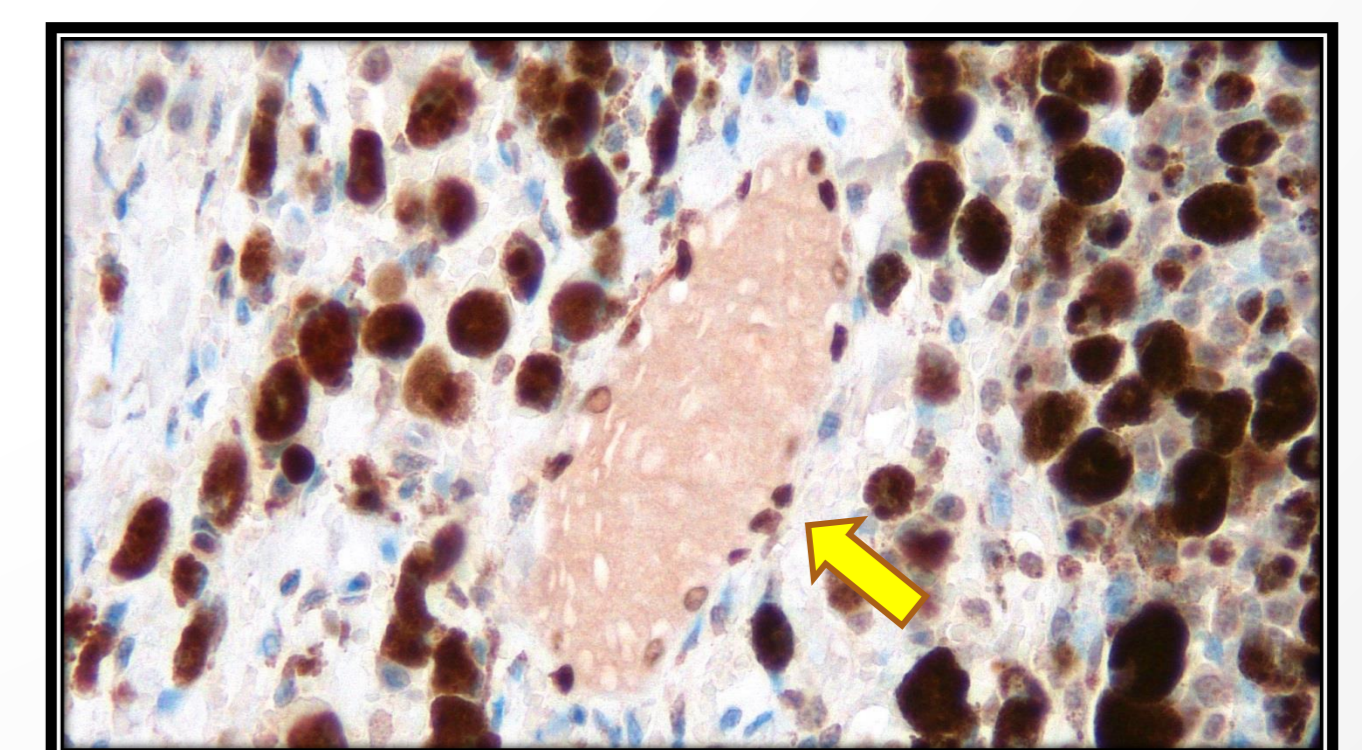
Next steps:

- Validate differential expression of selected genes by quantitative RT-qPCR.
- Perform RNA-Sequencing of 12 pairs of FFPE biopsies of primary oral melanomas and regional lymph node metastases.

Study B:

Project status:

- Identified 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.
- Optimised the immunohistochemical protocol for the lymphatic endothelial cell-specific marker PROX1 (rabbit anti-human, polyclonal, AngioBio, Del Mar, CA, USA) using FFPE canine tissue.
- Performed H&E staining and PROX1 immunohistochemistry on 20 primary oral melanomas.
- Independent, blind review of serial H&E and PROX1-stained oral melanoma sections by 3 pathologists.



Positive PROX1 nuclear signal in the central lymphatic vessel adjacent to melanoma cells in the adjacent tissue

Next steps:

- 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.