In Vitro and Histology Year Placement

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UNIVERSITY OF BATH

Introduction

RxCelerate provide leading drug discovery and development services all under one roof. Their cutting-edge toolkit provides the ability to unlock the true potential of a client's drug and deliver results quickly. Within RxCelerate, my placement was based within both the *in vitro* and the histology departments, assisting with both client and internal research. The *in vitro* team specialise in developing functional cell-based assays and immunoassays, which often serve as screening tools in early-stage drug discovery. The histology team analyse preclinical endpoints from a variety of tissues using immunohistochemistry (IHC) and tinctorial stains to validate *in vivo* disease models and evaluate compound efficacy.

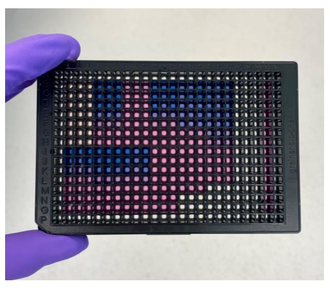
Techniques – *in vitro*

Tissue Culture

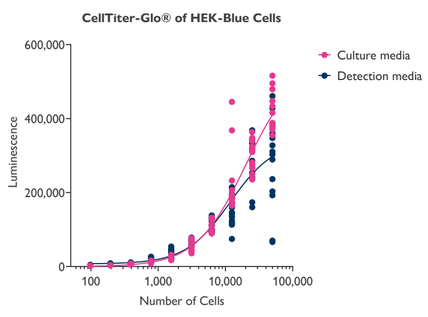
Culturing adherent and suspension cell lines. E.g. cells derived from human and mouse mammary carcinomas, lung cancers, and reporter cells.

Cell Based Assays

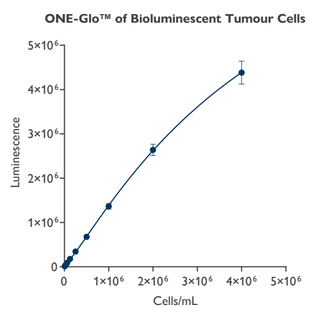
HEK-Blue Reporter Assay



CellTiter-Glo®



ONE-Glo™



Immunoassays

Western Blotting | ELISAs | Flow Cytometry

Techniques – Histology

Tissue trimming & processing in formalin and graded alcohols

Embedding in paraffin wax

Sectioning of FFPE blocks

Tinctorial Stains

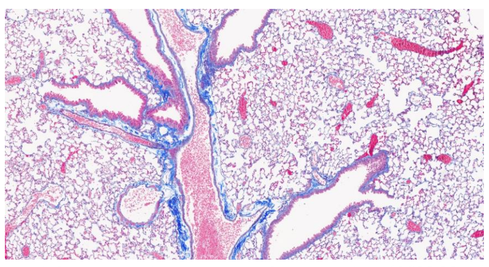


Figure 1: Masson trichrome stain on mouse lung section (10X Mag)

Immunohistochemistry Method Development

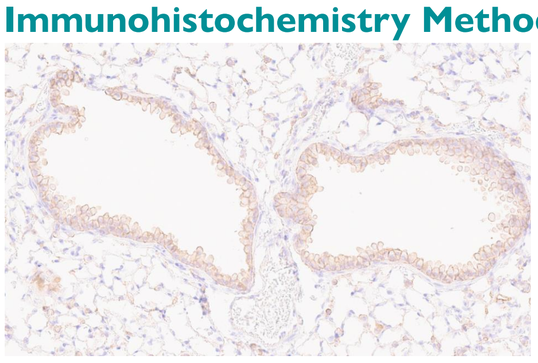


Figure 2: CK8 Immunohistochemistry on NSG mouse lung section (20X Mag)

Cytokeratin 8 (CK8) is an epithelial cell membrane marker. Within the lung, this IHC will stain airway epithelium and pneumocytes.

This also proves useful in identifying carcinomas. For example, cancer cells derived from mammary ducts will express CK8 because epithelial cells line the ducts in which they originated from.

Establishing Triple-Negative Breast Cancer Model

One internal research project I contributed to from both teams was the establishment of a triple-negative breast cancer model in mice. The aim of this project was to develop a model that mimicked the earlier stages of breast cancer, to eventually offer clients a pre-validated in-house model to test cancer drugs against.

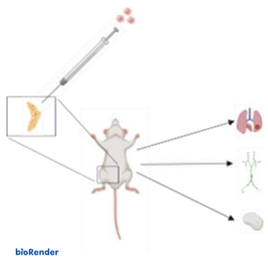
This internal study orthotopically grafted human breast cancer cells into the mammary fat pads of two strains of immunocompromised mice.

- NSG mice are amongst the most immunodeficient mice, lacking mature T cells, B cells, and natural killer cells.
- Athymic nude mice lack a normal thymus due to genetic mutation.

Methodology

1) Preparation of cells

Mycoplasma testing and harvesting into Matrigel.

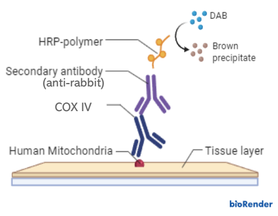


2) In Vivo Inoculation & Monitoring

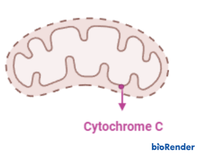
Tumour measurements with callipers and monitoring of body condition. Lungs, livers and lymph nodes taken

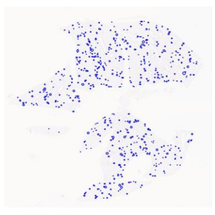
3) Immunohistochemistry

Lungs sectioned at 4µm, 5 levels apart for COX IV IHC.



Cytochrome C Oxidase IV (COX IV) is an enzyme localised to the inner mitochondrial membrane, specific to humans. This IHC will only identify the human cancer cells, not the surrounding mouse tissue.





4) Cell Counting

Scans of each stained lung level were analysed, and each single cell and cluster was counted manually.

Results

Early stages of breast cancer were successfully mimicked, with formation of a primary tumour at the inoculation site and spontaneous cell seeding to the lungs and liver. There were higher total cancer cell counts and tumour volumes in the NSG mice than the athymic nudes.

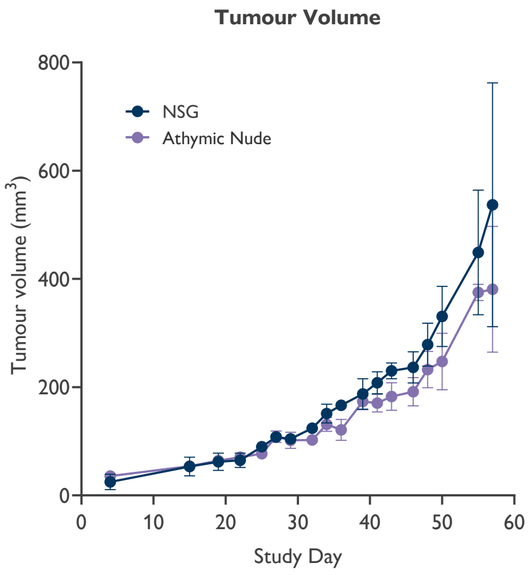


Figure 3: Tumour volume of NSG and athymic nude mice over time. *Callipers were used to measure the longest dimension of the tumour, then the width. Volume estimated using $V = xy^2/2$, where x is long dimension, y is width.*

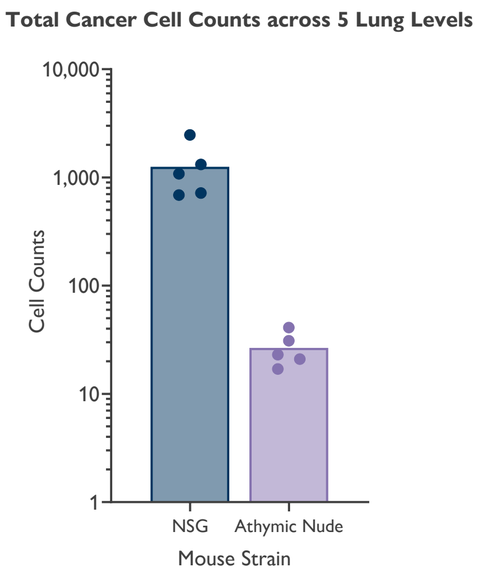
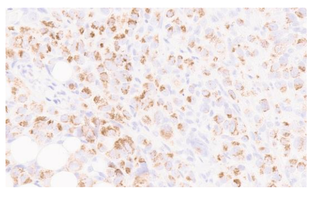


Figure 4: Total cancer cell counts (single cells and clusters) across 5 lung levels in NSG and athymic nude mice. *Means plotted, dots plotted as individual mice.*



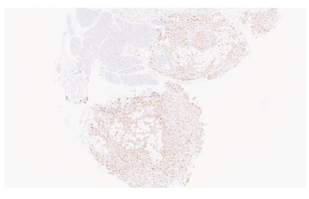


Figure 6: COX IV Immunohistochemistry on NSG mouse liver (40X Mag and 6X Mag). *Cancer cells are present in the adipose tissue.*

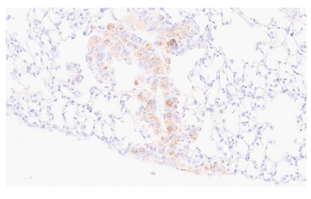


Figure 7: COX IV Immunohistochemistry on NSG mouse artery within the lung (25X Mag)

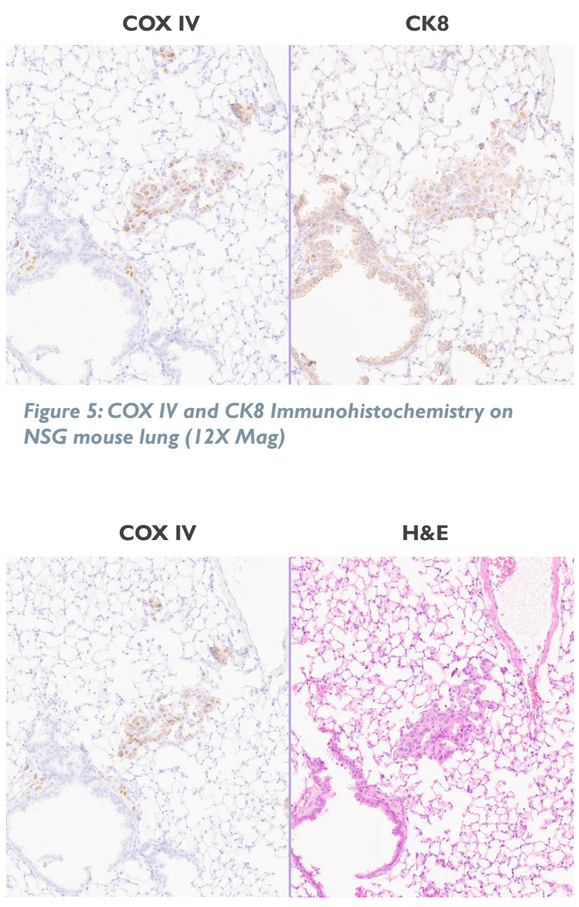


Figure 5: COX IV and CK8 Immunohistochemistry on NSG mouse lung (12X Mag)

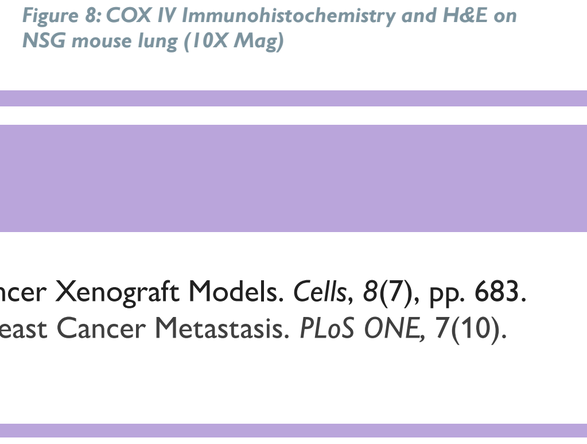


Figure 8: COX IV Immunohistochemistry and H&E on NSG mouse lung (10X Mag)

Acknowledgments and References

Cartoon illustrations from BioRender.com

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