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Cambridge

Ovarian Cancer

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My project was titled: **'The role of AMH/AMHR2 paracrine axis in modulating ovarian tumour microenvironment (TME) through regulation of cancer-associated mesothelial cells'.**

It has been shown that ovarian cancer cells can remodel mesothelial cells through secretion of AMH (antimullerian hormone) which acts on its receptor (AMHR2) on the mesothelial cells to modulate gene expression by downregulating canonical mesothelial markers and upregulating fibroblast like factors and anti-inflammatory cytokines.

Aims and objectives:

1) Validate results obtained on primary mesothelial cells in an immortalised mesothelial cell line (MPMCi). Previous experiments at this lab had shown that primary mesothelial cells are remodelled in the presence of gene expression. My aim was to confirm if this was also the case in MPMCi, so that this cell line can be used for future investigations.
2) Investigate interactions between various sources of mesothelial cells and KPCA (ovarian cancer cell line) spheroids.

Methods:

Validating results on primary mesothelial cells: I performed immunofluorescence, western blot, and qPCR to confirm whether similar patterns of gene modulation occurred when MPMCi were treated with conditioned tumour media as when primary mesothelial cells were treated.

Investigation of cancer spheroid / mesothelial interactions: I harvested omental and peritoneal cells from mice to create immortalised cell lines. I then plated these with KPCA spheroids. I also did the same on peritoneal cells with AMHR2ko, to see the specific effect of the AMH receptor in the remodelling process.

Through this experience, I was fortunate to learn a large range of wet-lab skills of mouse work, tissue culture, qPCR, RNA scope, western blot, cryo- and paraffin-section preparation, immunofluorescence (both on plated cells and slides). My project was one part of the larger project looking at how mesothelial cells get remodelled into cancer-associated mesothelial cells and eventually cancer associated fibroblasts. For

example, whilst my project focused on AMH, my supervisor looked at FSTL3 (follistatin like 3), another hormone secreted by ovarian cancer cells.

Results:

The results of my validation study did indeed corroborate results found in primary mesothelial cells, adding weight to their findings but also suggesting that the immortalised MPMCi cell line can be used as a model for further investigation. However, we had lots of problems getting the immunofluorescence result to work due to antibody concentrations added, hence I learnt the process of optimising antibodies.

When looking at the interactions of KPCA spheroids with mesothelial cells, we found that mesothelial cells did not stay at the edges but rather invaded into the centre of the spheroid.

<u>My views on histopathology and research</u>

The project was mainly focused on molecular pathology; however, we did use histopathology of human ovarian cancers to understand and classify the different types of epithelial cancers, influencing which cancer we modelled our cell line after. In the end we chose HGSOC (high grade serous carcinoma) to create the KPCA immortalised cell line from. We also had to classify the extracted ovarian tumours we received from the hospital in order to try to analyse the markers they expressed, which involved histopathology.

This has changed my view on research entirely. Before doing the lab placement, I just wanted to get insight into what bench research in pathology is like and was sure that I was only really interested in clinical medicine. I think this was largely down to never really understanding why I was doing what I was doing when performing laboratory skills in practical classes. However, I truly fell in love with science and research thanks to this placement: it gave me an appreciation for how different techniques are used to answer different questions in my project and gave me independence to think creatively about hypotheses in my project and how I may test them. This independence and grounding in science made research a lot more enjoyable and by the end of the project I was completely invested in what I was researching. I have continued staying in touch with the lab to help with some analysis remotely as I am so enthusiastic about the ovarian cancer research they are doing. Lastly, I now hope to have some aspect of research in my career in the future: whether that be histopathology or incorporating research through the career of an academic clinician.

I have not yet presented this work anywhere, however I am still in contact with my supervisor and am putting together a poster to present at the Cambridge University Oncology Society conference in February.