SRF VACATION SCHOLARSHIP REPORT 2017

Student's Name:	Chandler Bray	**Student's Institution/University:**	University of Sheffield
Degree Title and year of study:	Molecular Biology BSc		2/3
Supervisor's Name:	Dr Sarah Calvert	**Supervisor's Department and Institution:**	Department of Oncology and Metabolism at the University of Sheffield
Project Title:	Does a cell death protein regulate cell fusion in a placental cell line?		

Briefly outline the background and aims of the project *(max 200 words)*

My project was to determine if caspase-8 activation by Fas-ligand can induce cell fusion during placental cell differentiation. Caspases are proteolytic enzymes required for the breakdown of the internal cell structures leading to apoptosis, for example DNA fragmentation. These proteins can be initiated by external stimuli (e.g. Fas-ligand), consequently leading to the activation of the caspase cascade. However, recent theories have suggested caspases have an alternate role in cell differentiation.

The outer layer of the placenta is composed of two cell types; cytotrophoblasts and an outer multinucleated syncytiotrophoblast layer. Syncytiotrophoblast cells form a multinucleated mass known as the syncytium, which is essential for gaseous exchange between the fetal and maternal blood. Cytotrophoblasts are progenitor cells that undergo a dramatic differentiation process by losing their cell membranes in order to fuse into the syncytium. This fusion is essential to maintain the outer syncytiotrophoblast layer, but this morphological change is not completely understood at a molecular level.

I aimed to investigate morphogenesis of cytotrophoblasts to syncytiotrophoblast. The project was to induce the Fas-ligand signaling pathways in a cytotrophoblast cell line and observe whether this promoted cell fusion, therefore showing the caspase enzyme's ability to induce differentiation rather than cell death in the placenta.

Did the project change from that proposed in the application? If so, what changes were made and why? *(max 100 words)*

No extreme changes were made to the experiments. All reagents previously mentioned in the application were kept the same. However, I did use 3 different concentrations of Fas-ligand (25, 50 and 100ng/ml) to see the effect of lower than active apoptotic concentrations. Another change was that our DNA assay did not produce valid results, due to our apoptotic control not producing the expected DNA ladder, so therefore we analysed the cell morphology for qualitative data.

What were the main results/findings of the project? *(max 300 words)*

Despite the results of my DNA laddering assay being inconclusive I believe Fas-ligand was not inducing apoptosis in the BeWo placental line. This is because at a cellular level I saw physical signs of apoptosis in the Cisplatin samples, but not in the Fas-ligand samples. These apoptotic markers include cells rounding up and detaching from the plate and an uneven, unconfluent layer unlike the control samples, while Fas-ligand samples showed similar confluency to the control samples.

However, the apoptotic control, Cisplatin, did not show the expected DNA fragmentation markers. This could have been due to the speed to which we centrifuged the cells (10,000G for 5mins) so

that small fragments of DNA released to the media after apoptosis may have been lost from the assay or this could be due to DNA fragmentation being a late stage of apoptosis and therefore this 24hr incubation was not enough time to see this particular apoptotic marker.

Furthermore, using immunofluorescent data I could calculate the average percentage of nuclei in syncytialised BeWo cells. These were calculated by counting the number of nuclei in fused BeWo cells/total number of nuclei on 10 areas of each sample coverslip; finding the average percentage for each sample. This data was plotted with all 5 repeats, as shown in **Fig1.** The results suggest that low doses of Fas-ligand potentially induces cell fusion, even more than our fusion control, Forskolin. As I increased the Fas-ligand concentration the percentage of fused nuclei decrease. The data for the average of Cisplatin against both the average of Fas-ligand 25 and Forskolin showed significance with P values of 0.0216 and 0.0409 respectively*. However, these values were not significant from the Control, so further repeats must be undertaken to fully power this study.

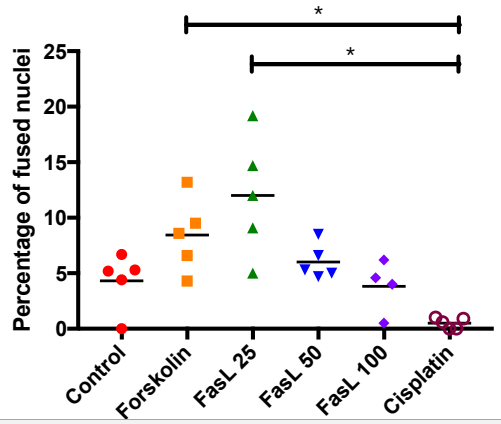

Fig1: graph showing the percentage of nuclei fused for the specific samples in each repeat and the average of all the repeats

What do you conclude from your findings? *(max 150 words)*

The DNA assay did not show laddering as expected, but cell morphology suggested apoptosis was triggered only in the Cisplatin cells. This data also gives preliminary indications that Fas-ligand at low doses may increase cytotrophoblast fusion, however, this will need further investigation.

I suggest that caspase-8 activation by Fas-ligand does increase the processes linked to cell differentiation and fusion in the placenta. Potentially, proteolytic caspase enzymes may be necessary to break down cytotrophoblast cell membranes during fusion into the syncytium. Therefore, further research may show increased levels of Fas-ligand may alter the rate of cell differentiation. As circulating Fas-ligand in maternal serum is increased in pre-eclampsia this could lead to increased cytotrophoblast turnover in this disease and contribute to the disease phenotype. Therefore further research is needed into the role of Fas-ligand in cytotrophoblast fusion and pre-eclampsia.

How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice? *(max 150 words)*

This experience has given me a greater understanding of how a research lab works and has strengthened my passion for the area of reproduction and fertility. To work with research professionals has opened by eyes to the word of scientific discovery and how better to organise my time in the future. My time during this project has given me a great insight into my project in the coming year and how to undertake the practical aspect, organising the literature and write up my work thoroughly.

Regarding the future of my studies, this has strongly influenced my decision to keep on with a scientific MSc degree after completing my undergraduate degree. However, my thoughts are directing me towards the clinical aspect of science, hopefully achieving a career in a practical scientific career that involves patient contact.

Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship *(max 100 words)*

Student:

The importance of the SRF Vacation Scholarship is extremely apparent; allowing people of a less experienced background to undertake a research led practical vacation is vital in skill development

and understanding what research entails. The scholarship allows funding for both the individual to live during the project and the vital funding for consumables during the project. The project was extremely enjoyable and something I'd like to see more students undertake in the future.

Supervisor:

The scholarship has been a great opportunity to pursue my own research ideas and allow me to develop as an independent researcher. The funding for consumables and a student have helped explore some ideas that may turn into fruitful scientific developments. Chandler has been an excellent student in the laboratory and I think this project will enhance his CV. Overall, this scholarship is good value to support a student with stipend and consumable budget. However, I ended up spending more than the consumables budget so £1000 consumables budget would more fully support a project of this nature.