SRF Vacation Scholarship Report

Investigation of the role of decidual macrophages in spiral artery transformation in early human pregnancy

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Main Aims

The original aim of the research proposal was to investigate the role of decidual macrophages in spiral artery transformation in early human pregnancy by examining the secretion of Ang1 and Ang2. Before the research began it was decided that it would be more suitable to investigate the decidual macrophages role via matrix-metalloproteinase (MMP) and urokinase-type plasminogen activator (uPA) both are involved in the degradation of the extra cellular matrix. MMP-2 and MMP-9 (the two gelatinases of interest) have been found to be involved in metastasis and can be linked to many types of cancer. Due to the ability of these enzymes to remodel tissue it is possible that they could be involved in the remodelling of spiral arteries directly or indirectly and allow/encourage later invasion of placental trophoblast cells. This is crucial for ensuring enough blood can reach and sustain the foetus and allow for a successful pregnancy. This project aimed to find if MMP-2, MMP-9 and uPA are secreted by decidual macrophages and whether this can be linked to extravillous trophoblast (EVT) cell invasion and thus spiral artery (SpA) remodelling.

Methods

We used zymography, using either a gel containing gelatin or casein/plasminogen, to determine the levels of MMP-2, MMP-9 and uPA produced by decidual macrophages, and whether the gestational age altered this production. We also investigated the effect of macrophage supernatants on the invasiveness of extravillous trophoblast cells. This involved extracting EVT cells from early placentas (8-10 weeks gestation) and incubating them on a Millipore filter with and without decidual macrophage supernatants. The cells which invaded were then stained using haematoxylin and eosin and counted. An invasion index (number of treated cells that invaded / number of control cells that invaded) was then calculated for each sample (n=8). The supernatants from these invasion assays were then used in further zymography to determine if the levels of MMPs and uPA were altered when macrophages were present and thus affect invasion. The analysis of the zymogram gels was done by using densitometry to calculate the intensity of the bands on each gel. This data was used to compare the levels of MMP and uPA in each of the samples.

The association between EVT cells and SpA remodelling was examined using immunohistochemistry of placental bed biopsies. Each SpA was marked and then examined using a scoring system to determine the degree of transformation. A more transformed vessel had almost no smooth muscle surrounding it and instead had fibrinogen around the circumference of the vessel.

Results and Discussion

This project provided a better understanding of how placental development is affected by the secretion of MMP-2, MMP-9 and uPA, also it produced conclusive evidence that MMPs and uPA are secreted by decidual macrophages. We found that the levels of uPA increased slightly when the EVT cells were treated with decidual macrophages, however this did not significantly alter the invasion. The effect of MMPs on invasion was also statistically insignificant. In this project only 2 MMPs were studied but there are many different MMP types which could possibly be secreted by decidual macrophages. Further investigation using the different MMPs would be beneficial as it is possible that other MMPs have different effects on EVT invasion and spiral artery remodelling. We can speculate that uPA involvement is important as it works via a complex pathway which involves inhibitors and activators. These inhibitors could have been present in this investigation and active which would prevent the uPA from affecting the EVT cells and thus prevent any affect the enzyme has on EVT invasion. To form a strong conclusion, further studies would need to asses the presence/secretion of these inhibitors and determine if they have an influence in preventing uPA from increasing EVT invasion. From assessment of the spiral arteries found in the decidua it was clear that remodelling was linked to the invading EVT cells which could be found in interstitial, intramural and endovascular locations.

I feel I have gained a wealth of invaluable experience from this project. I was taught important laboratory techniques such as basic tissue culture, immunohistochemistry and gel electrophoresis. I was also able to observe other techniques such as immunofluorescence and paraffin embedding as well as the use of specialist equipment in the bio-imaging department. I definitely feel that most of these techniques will be useful in many other areas of research. I was made more aware of the Human Tissue Act and the legal obligations of working with human tissue samples and

how to properly get consent for a project. I now feel I am better at organising my time and achieving set goals in a restricted time. My mathematical and statistical skills have also been developed by having to calculate concentrations and volumes and then analyse data from my results. By assessing spiral artery remodelling in placental bed biopsies I am now comfortable with histology and am experienced in using a microscope. The weekly research meetings demonstrated the importance of keeping good, accurate records of the project and any work that had been done or needed doing. From this I became more aware of how research is structured and how it often deviates from the original plan. This has lead to me being better at assessing work continuously, instead of waiting till all of it is complete. I am now also more patient and able to adapt to situations when they do not go as planned, for example having to alter aspects of an experiment to get the most reproducible results, and have learnt how to appropriately deal with anomalies.