

ELECTIVE (SSC5c) REPORT (1200 words)

A report that addresses the above four objectives should be written below. Your Elective supervisor will assess this.

Vascular dysfunction, including inappropriate angiogenesis, plays a fundamental role in the aetiology of many diseases, including two of the commonest ocular conditions of age-related macular degeneration (AMD) and diabetes mellitus. A key aspect of management strategies in such conditions is to monitor and retard dysfunction. For example, AMD management has been revolutionised by the advent of anti-angiogenic therapies based around anti-VEGF (anti-vascular and endothelial growth factor) antibodies. However, such treatments are beyond the healthcare resources of many countries, and a better understanding of this process will potentially lead to improved therapies, or treatments that do not have the prohibitive price tag of the current biologic agents. This elective was taken the form of a basic research project, where I began to develop a system to examine aspects of angiogenesis, in particular to explore the biology of signalling ligands implicated in the process. Our initial programme was to look at the responses of an eye endothelium preparation to the effects of ligands known to activate tyrosine kinase cell-surface receptors. We decided that a more interesting route would be to isolate the homologous molecules from a non-model system, *Crassostrea gigas*, with the goal of ultimately expressing them in a eukaryotic system and using these expressed proteins in endothelial assays, with the hypothesis that they may modulate the activation of the mammalian receptors in useful ways. I will detail the structure of the work in as I discuss objective 4, below.

Inappropriate angiogenesis in AMD can now be inhibited by the periodic injection of anti-VEGF antibodies into the eye, and this is what happens in the UK. There is no question that this is a highly efficacious therapy, and the preservation of quality of life that it engenders warrant its inclusion in the NICE guidelines. The price of these drugs is market driven, however, and at the current time, biological agents are extremely expensive, with annual costs per patients running to several thousand pounds, with these agents alone dwarfing the remaining pharmaceutical budget of eye hospitals. In large part this high price of biologics appears set by legal frameworks, rather than by the costs associated with drug development per se: the price of Avastin - a formulation of anti-VEGF antibodies licensed for intraocular injection in AMD - is 10-fold higher than off-label formulations used for other indications. One means by which healthcare systems can employ biologics therefore, is to use the identical agents incorporated into off-label formulations, and this is indeed what is now taking place in countries with fewer healthcare resources. Outside biologics, photodynamic therapy is commonly used to retard the production of angiogenic factors within the retina, thereby inhibiting neovascularisation. Although this approach works, and is many times less expensive than biological treatment, thus more easily incorporated into many healthcare systems globally, it has the downside of permanently destroying regions of retina. This is felt to be outweighed by the benefit of retaining vision, but an ideal therapy would clearly prevent neovascularization without destroying retina, but at a much reduced cost. It may be that an alternative approach is needed, where agents other than antibodies can influence the behaviour of the angiogenic endothelial cells.

Many would argue that vision is our primary means by which human beings obtain information about the world. A visual impairment, while typically non-fatal, can impact quality of life assessments as much as serious systemic diseases, including cancer, heart disease and stroke, resulting in a population with a considerably higher depression and suicide risk than non-visually impaired patients.

A full assessment of the visually impaired patient should therefore include not only an assessment of function (visual fields, acuity, reflexes, eye movements, etc), but also include an assessment on the patient's quality of life. In AMD and diabetic retinopathy, for example, the increased dependence on carers, increased risk of falls and reduced functional capacity all tend to lead to substantial decrements in quality of life assessments. This can be overlooked in clinical practice, where focus on the disease may come at the expense of focus on the patient. However, it is clear that quality of life issues must be addressed in any comprehensive management plan.

I hope that my future career will allow me to pursue both my clinical interests and my research interests. A main objective of this elective was to generate data that can be used as part of a publication, with which to expand and consolidate the research aspect of my portfolio. I trained previously as a cell and molecular biologist, and have many years experience in cell culture and recombinant DNA techniques, and have some experience of genetic analysis. However, there are several commonly used techniques that I did not have personal experience of, including epitope tagging, and ectopic gene expression in mammalian cells through the use of transfection platforms and mammalian expression vectors. I have now added these skills to my portfolio. In brief, we decided to isolate members from three gene families known to be involved in angiogenesis and vascular dysfunction, VEGF, fibroblast growth factor (FGF) and the insulin-related family of genes. We used the recently deposited genomic data from a non-model organism, *Crassostrea gigas*, to design PCR primers to isolate the gene orthologs. Once these PCR products had been verified, I reamplified employing primers that incorporated both an HA-tag to the 3'-end of the coding sequence, and restriction endonuclease adapters on either side of the PCR product, allowing me to perform a cohesive-ended ligation of the products into a mammalian expression vector backbone that we had chosen, IRES2-EGFP from Clontech. I was able to transfect HEK293 cells (a human kidney derived cell line) with these constructs, which could be readily assessed as transfected cells fluoresce green, due to the expression of the green fluorescent protein (EGFP) from the internal ribosomal entry site (IRES) in the construct. I was able to perform immunocytochemistry on the transfected cells, although this needs optimization before we feel confident that we have this working well in our hands. Our expectation is that the molecules we have chosen will be secreted, in which case a Western Blot of the supernatant of these cultures, when probed with an anti-HA tag antibody, should detect bands of the appropriate sizes. I feel that this objective has been met to a greater degree than I had hoped at the outset of the elective, and I feel that the work is now in good shape. The next steps involve assaying for function on cells of endothelial origin. Moreover, the VEGF homolog I isolated is most closely related to the human VEGF-C and VEGF-D; both implicated in lymphangiogenesis. It may be that the *Crassostrea* VEGF has a more potent modulatory effect on lymphatic endothelial cells as opposed to vascular endothelial cells, and this is something we would like to test. The past six weeks of the elective have been intense, highly productive (certainly from my standpoint), and very enjoyable. I have regained some of the confidence I had lost as a post-doctoral scientist prior to medical training, I have improved and developed my time management skills, and I feel in much better shape to combine these two aspects of my professional training, clinical and research, in my future career.