Zitzmann Lab Summer Internship Report – Ethan Prince

During the Summer of 2021, I had the opportunity to undertake a six-week research project in the Zitzmann Lab, within the Department of Biochemistry at the University of Oxford. I was incredibly fortunate to be under the supervision of a senior PhD student, Juliane Brun, who performed outstanding research on the Unfolded Protein Response (UPR) signature during Dengue virus (DENV) pathogenesis. To pursue this project, I was generously awarded funding from the Department of Biochemistry, as well as free accommodation and food from Merton College – to which I am immensely grateful for both. I would also like to thank Professor Nicole Zitzmann for allowing me to work in her laboratory, and Juliane Brun for equipping me with relevant laboratory skills and sharing her scientific expertise. As well, I would like to acknowledge the help of Snezana Vasiljevic, Mario Hansen and Bevin Gangadharan for enabling me to help with their projects to get familiar with a broad range of different scientific techniques.

Research is being conducted into the outcomes of dengue virus infection in macrophages by interfering with the UPR through CRISPR/Cas9 gene knock-outs using gRNAs. Transcriptomic data obtained in the lab and previous literature suggest the UPR is relevant for dengue infection, but it is not yet fully understood. Dengue has a huge economic impact on affected countries, as there is no test to predict the severity of disease. Unpublished patient serum data derived from the Zitzmann lab suggest that a specific early UPR signature could potentially be used to predict the severity of outcome of the viral infection. The aim of the project is to help to understand this UPR signature, by determining which of the three main arms of the UPR is the most important in dengue pathogenesis, and by increasing the efficiency of existing protocols to study this.

Rationale Behind the Experimental Set-Up:

The problem with DENV infection is not necessarily the virus itself, but the response to the infection by the immune system in the form of cytokine storms. This is seen in a separation of the peak viral load in blood serum with peak hospital admissions of the same patients. Patients admitted to hospital may not be expected to accurately test DENV-positive.

The UPR has been implicated in DENV pathogenesis, and may be used as a clinical marker to predict whether someone may require hospitalisation prior to admission (e.g., when testing a close-contact of an infected person). A large target of DENV infection are macrophages, but they are non-dividing cells, so one cannot do knock-outs on them. Therefore, iPSCs were produced from dermal fibroblasts, where knock-outs using gRNAs delivered by transfection reagents were performed, and the iPSCs were re-differentiated into mature macrophages. These macrophages were then infected with DENV.

The secretion of dengue virions can be measured via a plaque assay, a focus-forming units (FFU) assay or qRT-PCR. A plaque assay measures the number of cells lysed by infectious virus particles. An FFU assay measures the titre of infectious virus by immunostaining (which is more useful for classes of virus that do not lyse cells and hence do not plaque easily). The qRT-PCR method will measure the amount of viral RNA (although this does not necessarily mean that it is infectious!).

One aim of the project was to verify a new, high-throughput plaque assay protocol to stream-line research into this UPR signature. This involved performing the old protocol in parallel with the new method to ascertain whether the results were comparable, and hence whether the new protocol could be verified. I also validated that the iPSCs had the desired UPR genes knocked-out by DNA analysis (PCR using primers both flanking the deletion regions and inside the deletion regions, followed by gel electrophoresis and confirmatory DNA sequencing show in figure 1 and 2) and protein analysis (to see whether nonsense-mediated decay occurred properly) via Western blotting.

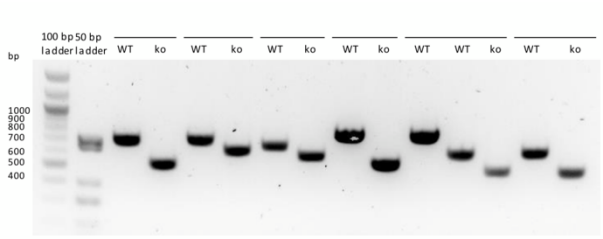

Figure 1: A DNA gel showing the effect of the knock-outs in the genes of interest. Primers flanking the deletion region were used, producing bands of a longer length if the knock-out was unsuccessful, or bands of a shorter length if it was successful.

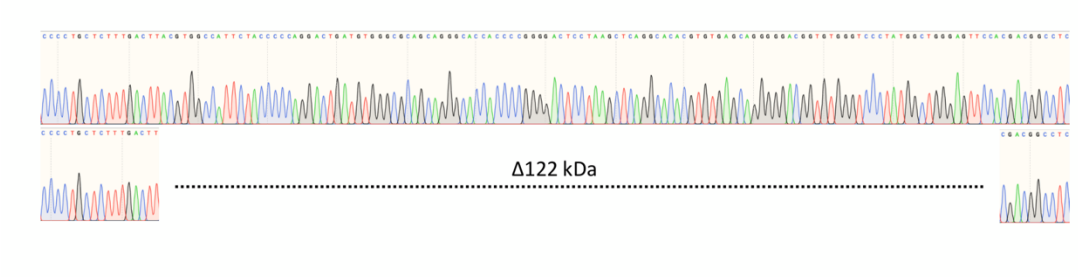

Figure 2: Sequencing data obtained for the WT and knock-out cells for a gene called MOGS. The second line shows the deletion region, confirming that it had indeed occurred.

Laboratory Skills:

My practical skillset has significantly expanded during the course of this summer project. I am now more confident with techniques such as cell culturing cancer cell lines and iPSCs, PCR, Western blotting, Southern blotting, in-gel trypsin digestion for mass spec, ELISA, protein purification on affinity and SEC columns, MaxiPrep, as well as others. A large part of scientific research is troubleshooting experiments – to which I also now have experience (e.g., identifying that a plaque assay was unsuccessful as the viral stock used to infect the cells was inactive). Furthermore, I now have exposure to working in both containment level 2 (CAT2) and containment level 3 (CAT3) laboratories.