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RE: Report for First Contact Initiative Grant 2019

Dear ESC Council on Basic Cardiovascular Science,

Firstly, I would like to sincerely thank Professor Johannes Waltenberger and the ESC Council for awarding me 2019 First Contact Initiative Grant. This gave me the invaluable opportunity to visit the laboratory of Dr. Borja Ibanez at The Centro Nacional de Investigaciones Cardiovasculares (CNIC) in Madrid, Spain. The visit took place from the 20th October until the 18th of November 2019.

My postdoctoral research at Queens University Belfast, in the laboratory of Dr Chris Watson, is focused on investigating the role of epigenetic regulation in cardiomyopathies with a keen interest in mechanisms that are involved in ischaemic injury and exposure to chronic hypoxia. Ischaemic injury, as a consequence of myocardial infarction (MI) induces death and remodelling of the underlying tissue, including infarcted area as well as surrounding viable myocardial tissue. The ischaemic heart is particularly characterized by an overactive fibrotic phenotype resulting from the activation and proliferation of resident fibroblasts to myofibroblasts with enhanced secretion of extracellular matrix proteins, promoting scar formation. Clinical consequences of ischaemic remodelling include the development of ventricular dysfunction, arrhythmias, and the development of cardiac failure. Our preliminary data has found that hypoxia-induced pro-fibrotic changes in human cardiac fibroblasts from a healthy donor are associated with global DNA hypermethylation and increased DNA methyltransferase expression. Furthermore, using GeneChip human promotor tiling array analysis of these human cardiac fibroblasts cultured in response to chronic hypoxia, I have identified 133 gene-specific DNA hypomethylation and 37 gene-specific DNA hypermethylation events. The influence of these epigenetic changes on gene regulation in response to hypoxia are being validated *in vitro*, however whether these ischemia-driven alterations in DNA methylation in cardiac fibroblasts occurs *in vivo* remains to be investigated.

In order to pursuit this research objective, we wanted to investigate these changes in a murine model of MI. This model is induced by surgical ligation of the left anterior descending (LAD) coronary artery in order to induce myocardial ischaemia and subsequent pathological cardiac remodelling.

LAD coronary artery ligation in a mouse is a technically challenging surgical procedure and while it is well established internationally, there are currently no research groups in both Ireland and Northern Ireland carrying out this procedure. In order to train in this model, I contacted Dr. Borja Ibáñez, principal investigator of the Translational Laboratory for Cardiovascular Imaging and Therapy at the CNIC, Madrid. Dr. Ibáñez is a leading researcher in myocardial diseases with a primary research interest in ischemia/reperfusion (IR) injury and heart failure and his research group have established models of MI in both rodents and large animals and routinely carry out the LAD ligation surgical procedure.

During my visit at the CNIC, I was very lucky to receive the best possible technical advice from Dr. Ibanez' research group, especially with regards to surgically inducing ischemia/reperfusion injury and permanent LAD ligation surgery. Under the patient and expert guidance of Monica Gomez, I became proficient at inducing permanent surgical ligation of the LAD to induce MI in mice. Initially, training started off visualization of both IR and permanent ligation from Monica, followed by practical hands-on training in non-recovery. As my skills and surgical proficiency showed improvement, I then progressed to recovery procedures. By the end of the visit, I performed 22 recovery procedures and achieved a post-surgical survival rate of roughly 63%. As part of my visit, I was also trained in quantifying both the area at risk and infarct size by Evans blue and TTC staining of murine hearts post ischaemic injury.

While over at the CNIC, Dr. Ibanez also provided me with access to histological sections and snap frozen left ventricular tissue samples from a previous IR study performed in a porcine model (45 days post injury, both from the infarct area and the remote area) that had established cardiac dysfunction associated with injury (assessed by MRI). Global DNA methylation in these tissue sections was examined by nuclear immunohistochemical staining for 5-methylcytosine (5MeC) and with positive pixel quantification, we found that 5MeC levels were significantly increased in tissue form the infarct area compared to the remote cardiac tissue. RNA was also isolated from these tissue samples using the RNEasy Fibrous Kit (Qiagen) followed by cDNA synthesis (Applied Biosystems). I also designed and optimized porcine primers for the three DNMT enzymes along with some of the candidate genes used in our hypoxic human fibroblast methylation analysis. Unfortunately, during primer optimization, those designed for the fibroblast candidates were found to be non-specific for an efficient qPCR and could not be used. Those designed for the DNMT enzymes were determined as specific and we found that DNMT3B is significantly upregulated in the infarcted myocardium and demonstrated a significant inverse relationship with cardiac function (ejection fraction). These results mirror our previous findings in human fibroblasts exposed to chronic hypoxia. Regarding mRNA levels of DNMT1 or DNMT3A, we found no difference between remote and infarcted porcine

myocardium. Upon returning to Queens University, we recently received ethical approval and had initially started to perform some of the surgeries in order to set up the model in Belfast. Unfortunately, due to the COVID-19 outbreak, this work has been subsequently placed on hold until the pandemic subsides. It is our interest to subsequently investigate the role of these differentially methylated genes highlighted from fibroblasts in the context of ischaemic injury.

In summary, the CNIC Madrid was an ideal place to train and conduct research. The research environment was amazing with state-of-the-art equipment specifically enabling high-class cardiovascular research. The CNIC has a warm and vibrant atmosphere with a collection of helpful and friendly research and administrative staff present, which was the reason I was able to confidently leave Madrid, expertly trained in the LAD ligation surgical technique. Other than the experimental work, we collectively discussed the exciting future collaborative studies between Queens University and the CNIC Madrid.

Finally, I would like to sincerely thank Dr. Ibanez and his amazing research group, especially Dr. Eduardo Oliver Perez, Monica Gomez, Rocío Villena, Agustín Clemente, Valeria Lalama, Dr. Susana Rocha and Dr. Carlos Galán for making my trip to the CNIC such a fantastic and unforgettable experience. I would also like to reiterate my sincere gratitude to the ESC Council on Basic Cardiovascular Science for this trip would not have been possible without their support.

Yours sincerely,

Adam Russell-Hallinan