Pump-Priming Grant

Report

Grant Recipient: Fungai Dengu, Clinical Research Fellow

DPhil Student (Nuffield Department of Surgical Sciences)

Title: Characterisation of the molecular & immunophenotype of human donor livers during normothermic machine perfusion (NMP) in liver transplantation

Objective: The overall aim of this study was to evaluate the impact of prior cold ischaemia on the proteomic and molecular profile of NMP livers.

Questions:

1. What is the effect of NMP on the liver proteome? Longitudinal analysis through the different phases of 'back to base' machine preservation (static cold storage, normothermic machine perfusion and reperfusion
2. What are the underlying pathways and mechanisms involved in NMP? Downstream analysis of pathways related to the proteins identified.

Methods

Liver tissue samples (n=57) representing the end of cold storage (LT1), the end of NMP/total preservation (LT2) and in situ organ reperfusion (LT3), were analysed from a prospective clinical trial of 'Back to Base' NMP. Following homogenisation and protein extraction, samples were digested and analysed by quantitative label-free LC-MS/MS (timsTOF Pro, Bruker). Longitudinal comparisons of LT1, LT2 and LT3 were performed and comparisons of livers with shorter (<6hrs) or longer (>6hrs) CIT were also made at LT2 and LT3. Protein–protein interaction (PPI) networks and functional enrichment analysis were performed using the STRING database.

Results

Over 6000 proteins were identified in the sample's library. The Principal Component Analysis (PCA) demonstrated grouping of the samples according to the timepoint of the samples. In keeping with this, there were 110 differentially expressed proteins at LT2 vs LT1. 24 proteins were differentially expressed in livers with short vs long periods of cold storage prior to machine perfusion when comparing protein expression at the end of preservation. Proteins involved in maintaining cellular homeostasis and removal of damaged or misfolded proteins were significantly downregulated at LT2 in livers with short SCS compared to long SCS. The differently expressed proteins at LT3 were associated with autophagy and cell-cycle regulation.

Outputs (publications/presentations)

International Presentations

Oral: European Society of Transplantation (ESOT) biennial International Congress 2021 (Milan)

Proteomics profiling of molecular changes during normothermic machine perfusion of the liver. M. L. Lo Faro, A. Thorne, H. Huang, M. Kaisar, S. Davis**, F. Dengu**, S. Shaheed, J. Mulvey, R. Fischer, D. Nasralla, A. Santos Delgado, B. Kessler, H. Leuvenink, P. Friend, R. Ploeg on behalf of the COPE consortium

Brief Oral/Poster: European Society of Transplantation (ESOT) biennial International Congress 2021 (Milan) , ESOT 2021

Prolonged cold storage prior to Normothermic Machine Perfusion alters the molecular profile of donor livers: insights from 'Back to Base' liver NMP.

F. Dengu, A. Thorne, M. L. Lo Faro, H. Huang, S. Shaheed, M. Kaisar, S. Davis, J. Mulvey, R. Fischer, C. Ceresa, D. Nasralla, A. Santos Delgado, B. Kessler, H. Leuvenink, P. Friend, R. Ploeg

Local Presentation:

Nuffield Department of Surgical Sciences Away Day 2020, Selected for Full Oral Presentation.

Molecular and proteomic signatures associated with preservation related graft injury: insight from human liver normothermic machine perfusion (NMP).

F. Dengu, A. Thorne, M. L. Lo Faro, H. Huang, S. Shaheed, M. Kaisar, S. Davis, J. Mulvey, R. Fischer, C. Ceresa, D. Nasralla, A. Santos Delgado, B. Kessler, H. Leuvenink, P. Friend, R. Ploeg

Other Media & Communication:

Profile and Article in Trinity College Alumni Newsletter – with over 500 social media reads (2021)

Next Steps (what is it leading to)

This work has been critical in progressing our understanding of the mechanistic effects of normothermic machine perfusion and establishing a multi-omics appreciation of what is occurring to the liver graft during different permutations of this novel preservation method (i.e back to base vs device to donor).

The next steps will be to use this newly developed database of protein signatures (linked to clinical conditions and outcomes) to perform hypothesis driven research on NMP in liver transplantation. Novel therapies targeting pathways and proteins identified in this work to be associated with worse outcomes or greater injury are the starting point but also non pharmacological interventions are being tabled that can assessed against this invaluable and unique proteomic database.