Pump-Priming Grant

Report

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Collaborators: Gabriel Oniscu (Edinburgh) in collaboration with Evotec

Title

NRP: a technique to repair and recover DCD kidneys.

Objective

Normothermic Regional Perfusion (NRP) is an organ retrieval technique that acts as a perfusion bridge between asystole and retrieval allowing enhanced assessment of organs while reducing ischaemic injury. During warm ischemia, ATP degradation leads to the progressive accumulation of xanthine and hypoxanthine, important sources of superoxide free radicals at organ reperfusion. Post-ischaemic NRP in donation after cardiac death (DCD) donors should restore cellular energy substrates, reduce levels of nucleotide degradation products, and improve the concentrations of endogenous antioxidants to stimulate cellular repair. This reversal of anaerobic metabolism by replenishing mitochondrial stores of ATP, mimics ischaemic preconditioning although the exact pathways are unknown.

While it is well recognised that NRP livers offer improved transplant survival compared to standard donation after cardiac death (sDCD) livers, kidney transplant data is less clear but recent updates support an improvement in function with limited evidence of harm. However, the variation in global NRP and DCD techniques make it difficult to directly compare studies.

Our hypothesis was that NRP-DCD kidneys undergo molecular changes during NRP that impact on outcome when compared to sDCD and in this regard, NRP kidneys are anticipated to have more in common with brainstem death (DBD) or live donor (LD) kidneys. We proposed profiling the tissue transcriptome of NRP kidneys and compare to deceased donor and live kidney cohorts to identify molecular changes indicative of recovery.

Method

Kidney biopsies from live donors (n = 20), donors after brainstem death (DBD, n = 20) or cardiac death with static cold storage (sDCD, n = 20) or normothermic regional perfusion (NRP-DCD, n = 20) were obtained from the QUOD biobank and processed for RNA sequencing. The samples were matched with comparative baseline characteristics based on donor age, functional warm ischaemic time (fWIT= time when systolic BP drops <50mmHg after withdrawal treatment) and donor creatinine at retrieval. The donors were chosen to ensure that they had renal tissue in RNALater and FFPE blocks with urine and serum samples also available for further studies.

From these 73 transcriptomes were identified and 66 available for analysis after quality control. Transcriptomes were clustered by similarity via self-organizing maps (oposSOM)

and further explored via dimension reduction (PHATE) to integrate technical, clinical and molecular data. This was performed by Evotec, a Biotech company in collaboration with QUOD and combined with DBD and sDCD samples from other projects to better assess tissue characteristics. Evotec undertook unsupervised analysis of biopsy transcriptomes to identify discernible kidney injury and repair pathways for further focused study.

Results

Unsupervised clustering of biopsy transcriptomes revealed three molecularly distinct groups of different sizes (Fig. A), labelled blue (n = 724), red (n = 170) and green (n = 75). Interestingly, molecular clustering did not separate DBD from DCD, highlighting molecular similarities largely independent of donor type (Fig. B). Integration of clinical and technical variables using a decision tree approach suggested that red and green clusters could be explained by RNA quality and biopsy type (core needle vs. punch), respectively. RNA concentration showed a gradient separating the red cluster from high-quality samples (Fig. C). Variability in sampling procedures affected biopsy composition, with the green cluster enriched for needle biopsies and characterised by a stronger medullary signal (Fig. D). Importantly, initial exploration of high-quality transcriptomes with comparable tissue composition (cluster blue) revealed gradients of tubular damage and metabolic adaptation, and stromal and immune activation, underlining the potential of the dataset to study mechanisms of kidney injury and repair.

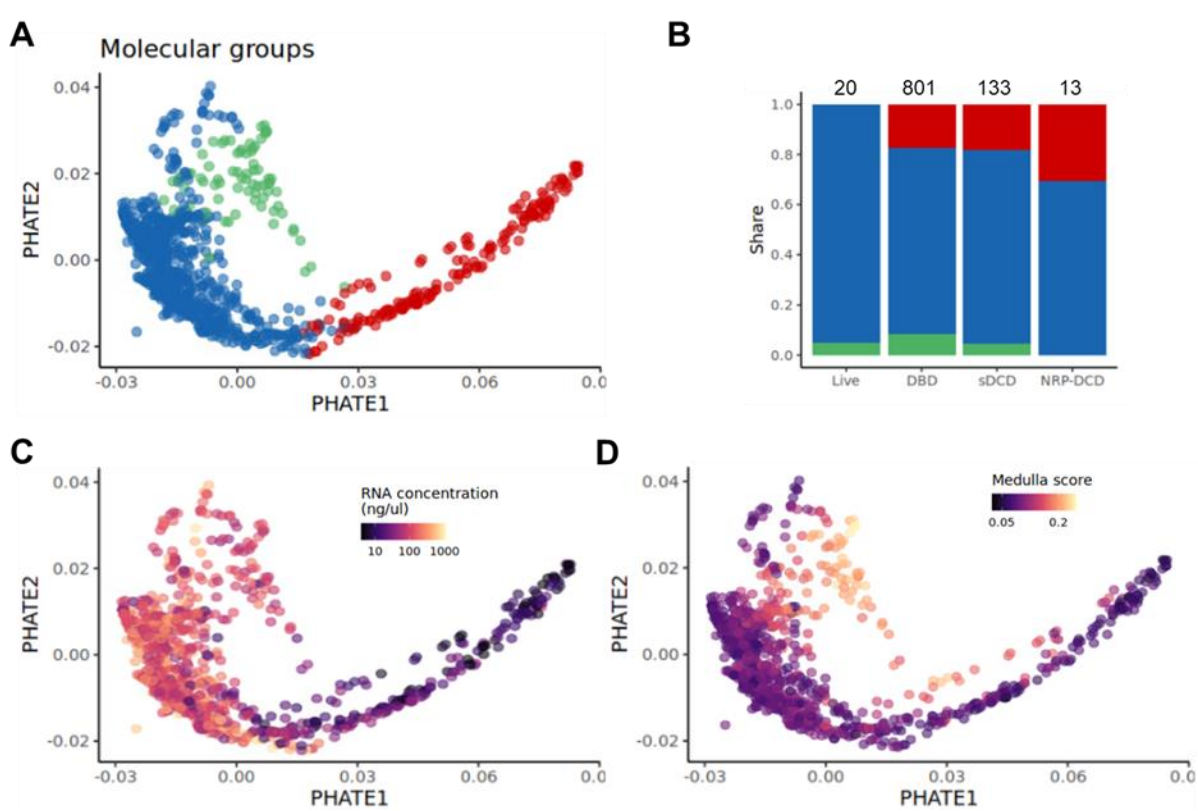

Figure 1: Unsupervised analysis of donor kidney biopsy transcriptomes revealed gradients in RNA quality and tissue composition. (A) Biopsy transcriptomes were clustered into three molecular groups using self-organising maps. Groups were arbitrarily labelled blue, red and green, and PHATE dimension reduction revealed a global data structure separating the red and green clusters from the blue cluster on dimensions one and two,

respectively. (B) Distribution of donor types across molecular groups, with DBD and DCD transcriptomes contributing comparably to all three molecular groups. (C) Embedding sample RNA concentration and integrity (not shown) in PHATE space revealed a gradient separating the red cluster from other transcriptomes. (D) Embedding a medullary gene expression signature in PHATE space separated the green cluster from the blue and red clusters, consistent with the over-representation of core needle biopsies (not shown) resulting in a higher medullary content in this molecular group.

Further analysis has now moved to focusing on differences in injury pathways such as reactive oxygen species (ROS), complement and hypoxia between the groups.

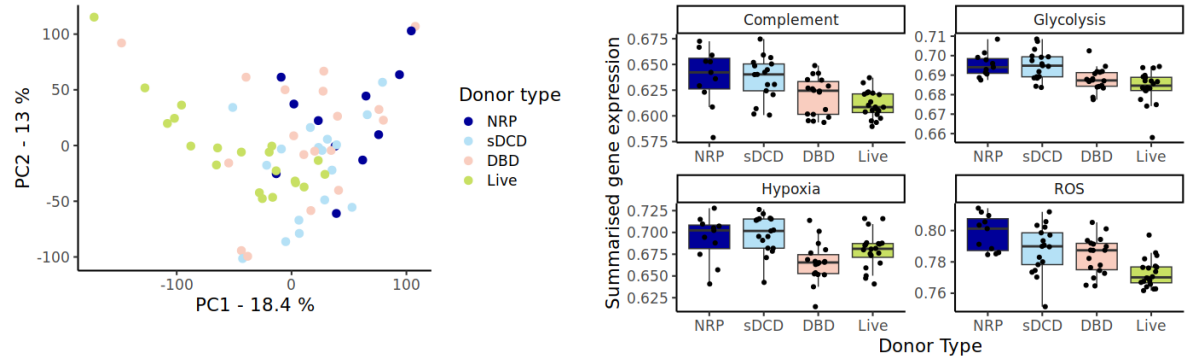

Figure 2. Results from unsupervised analysis of biopsy transcriptomes identifying genes from various IRI pathways.

Outputs (publications/presentations)

Poster presented at ESOT 2023 Athens

Unsupervised analysis of kidney biopsy transcriptomes from donors after brainstem or cardiac death. Rachel Thomas, Tobias Bohnenpoll, Ricardo Castro, Maria Kaisar, Maria Letizia Lo Faro, Georg Ebeling, Sadr Shaheed, Lente Lerink, Nelly Mostajo Berrospi, Rayan Daou, Tim Blokker, Olivier Radresa, Uwe Andag, Gabriel C Oniscu, Rutger Ploeg.

To highlight that unsupervised analysis of biopsy transcriptomes revealed technical variability in RNA quality and tissue composition, potentially confounding biological signals. Data-driven selection of high-quality transcriptomes with comparable tissue composition will enable the study of kidney injury and repair mechanisms across major transplant conditions.

Next Steps (what is it leading to)

We continue to work on this project after issues with Evotec sample analysis capabilities. Further investigation has now started including group-based differential gene expression and functional enrichment analysis and in-depth transcriptomic characterisation with additional potential to find predictive correlates of transplantation outcome.

Since the project restarted in December 2023, the team now meet monthly to receive updates from our analysis collaborative partners Evotec with us providing clinical context. Evotec has asked us to identify genes of interest for more focused analysis and Dr Kaisar and Dr Lo Faro

and I will meet next week to do this. We will then all meet again in April. The aim at that meeting is to have a more detailed analysis from our unique dataset, which can be presented internationally and published. This work is also being explored in Oxford from a proteomics and metabolomics perspective and duplicated in liver tissue to better explore and characterise NRP impact on tissue.

Rachel Thomas

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