Comparing gene expression and pathways between human and porcine lung models of *ex vivo* lung perfusion

Adrian P Karman (200769954), Jenny Gilmour, Simi Ali
Translational and Clinical Research Institute,
Faculty of Medical Sciences, Newcastle University
a.p.karman2@ncl.ac.uk

I. Introduction

- **Lung transplantation** is the only effective option for patients with end-stage lung diseases when all conservative treatments have failed. However, there is a glaring discrepancy between the availability and demand for suitable lung donors, increasing the likelihood of patients dying before a donor becomes available.[1]

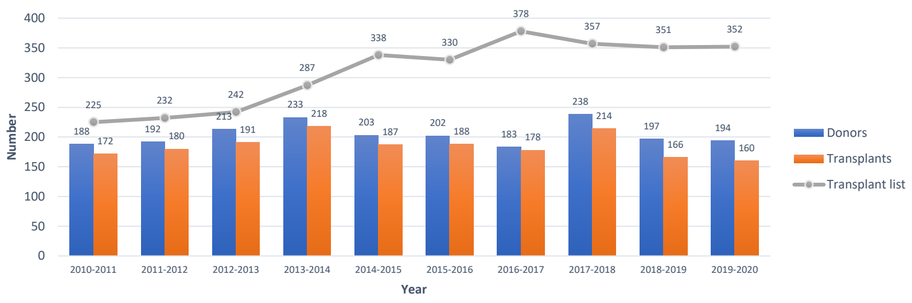


Figure 1: Lung and heart-lung transplant list in the UK, 2010 – 2020. The number of patients on the transplant list (grey line) show an upward trend while the numbers of donors and procedures have not been able to keep up. Adapted from data by NHS Blood and Transplant.[1]

- **Ischaemia-reperfusion injury (IRI)** leading to **primary graft dysfunction** is one of the main causes of early morbidity and mortality post-transplantation, characterised by a complex inflammatory process involving the activation of immune cells, oxidative stress, and endothelial dysfunction.[2]

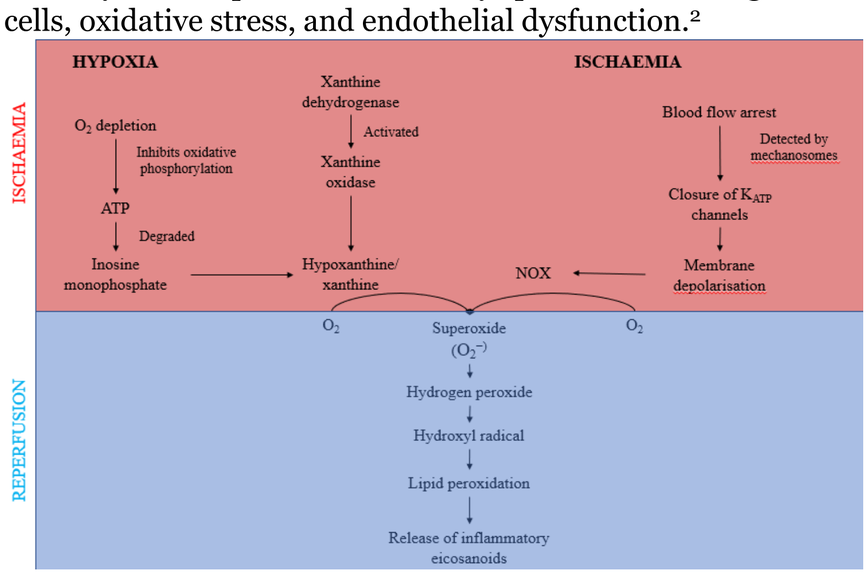


Figure 2: Pathophysiology of IRI. The production of ROS causes most of the damage during reperfusion. Both hypoxic and ischaemic conditions contribute to the production of ROS.[2]

- **Normothermic *ex vivo* lung perfusion (EVLP)** preserves, assesses, and reconditions marginal lungs that were initially deemed unsuitable for transplantation, thereby increasing the donor pool and mitigating the incidence of IRI. Lungs are perfused with a hyper-oncotic solution (Steen) which creates the osmotic pressure needed to maintain physiologic perfusion.[3]

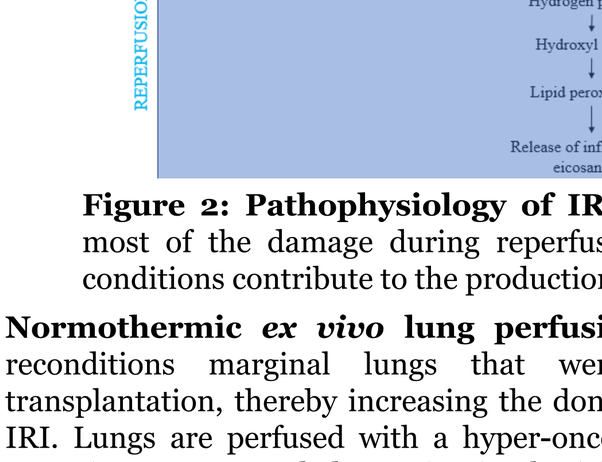
II. Aims

1. Identify the differential genes and pathways expressed post-EVLP in porcine lungs.
2. Compare the genes and pathways with results from human EVLP models.

III. Methods

Porcine post-EVLP data

- Lung biopsies performed by XVIVO Perfusion
- Differentially expressed genes (DEGs) identified using RNA-sequencing



Human post-EVLP data

- Microarray data from GEO database by Wong *et al.*[4] (GSE127055)
  - n=49, 5 hours EVLP
- RNA-sequencing data from study by Ferdinand *et al.*[5]
  - n=10, 4 hours EVLP

Analysis of Gene Ontology and Pathways using DAVID tools

Analysis of Protein Association using STRING tools

Statistical Analysis

- Volcano plots using GEO2R and R
- Heat maps using GraphPad Prism

RNA isolation

Reverse transcription to cDNA

Real-time polymerase chain reaction for IL-6 and TNFAIP3

IV. Results

1. **RNA-sequencing gene expression profiles in EVLP models are distinct between the two groups**

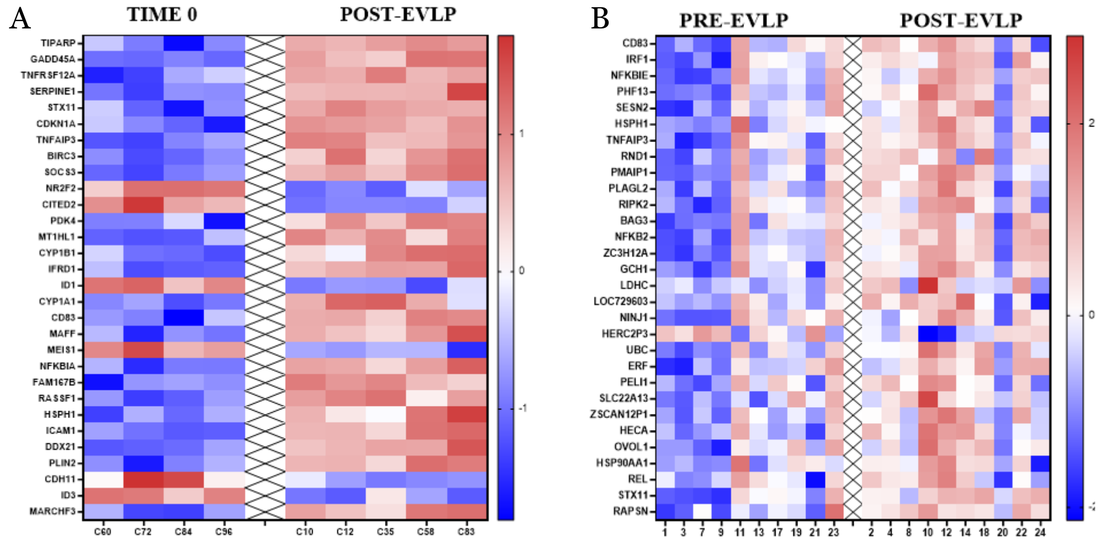


Figure 3: Transcriptional changes occur during EVLP. Heatmaps of the top 30 most significant DEGs in A) Porcine and B) Human EVLP. Numbers in x-axis are sample names, while the y-axis are gene IDs. Colours depict the value of z-scores; red indicates higher values while blue indicates lower values.

2. **Significant upregulation of IL-6 and TNFAIP3 post-EVLP**

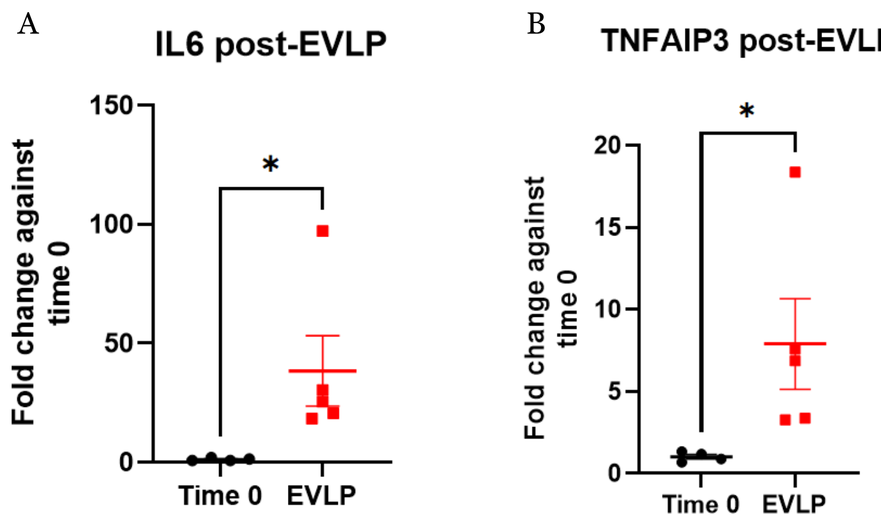


Figure 4: IL6 and TNFAIP3 are upregulated following porcine EVLP. Fold change of A) IL-6 and B) TNFAIP3 relative to time 0. RNA was extracted from lung biopsies and expression was assessed using RT-qPCR. Fold change relative to time 0 was calculated using the delta-delta Ct method and normalised to beta actin. Error bars represent the standard error of the mean. Statistical significance was calculated using an unpaired t-test (* $p < 0.05$).

3. **There are 3 notable pathways involved in immune processes that overlap in porcine and human EVLP models**

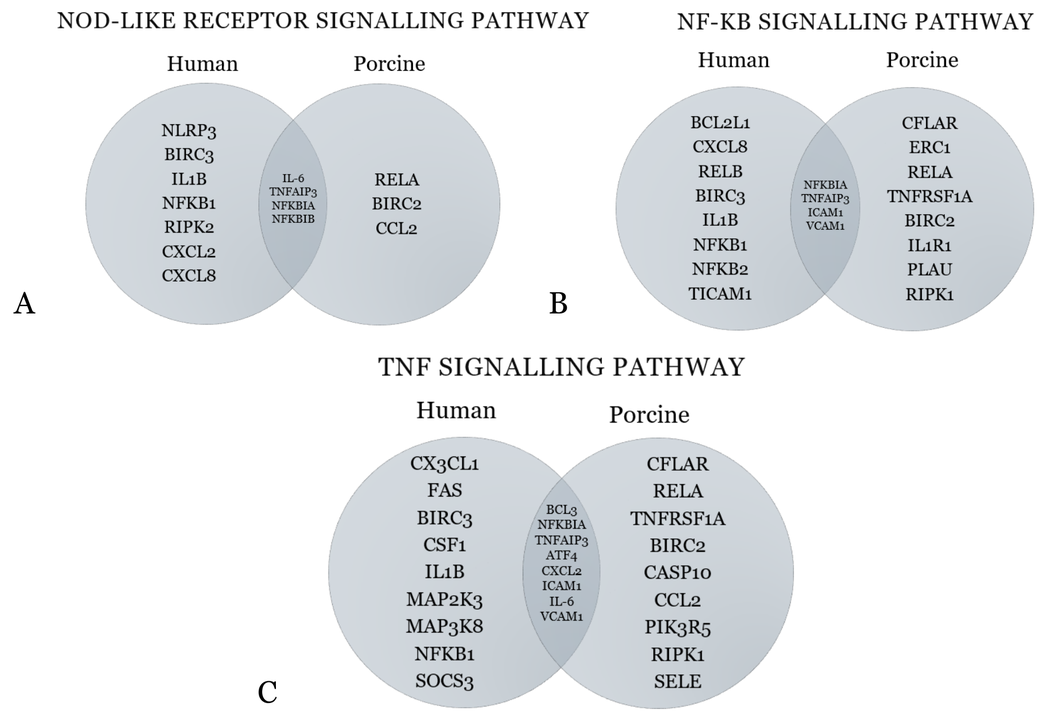


Figure 5: Overlapping genes in each pathway. A) 4 in NOD-like receptor, B) 4 in NF-κB, and C) 8 in TNF signalling pathway.

V. Conclusion

There is distinct expression of genes and pathways following EVLP, mostly involved in immune processes. Furthermore, comparative analysis between porcine and human EVLP models show overlapping genes and pathways. These findings support the use of EVLP as a method to increase the donor pool.

REFERENCES

1. NHS Blood and Transplant. Organ donation and transplantation activity report 2019/20 [Internet]. 2020 May [cited 2021 Jun]. Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/19481/activity-report-2019-2020.pdf
2. Chatterjee S, Nieman GF, Christie JD, Fisher AB. Shear stress-related mechanosignaling with lung ischemia: lessons from basic research can inform lung transplantation. Am J Physiol Lung Cell Mol Physiol. 2014 Nov 1;307(9):L668–80.
3. Pan X, Yang J, Fu S, Zhao H. Application of ex vivo lung perfusion (EVLP) in lung transplantation. J Thorac Dis. 2018 Jul;10(7):4637–42.
4. Wong A, Zamel R, Yeung J, Bader GD, Dos Santos CC, Bai X, et al. Potential therapeutic targets for lung repair during human ex vivo lung perfusion. Eur Respir J. 2020 Apr;55(4):1902222.
5. Ferdinand JR, Morrison MI, Andreasson A, Charlton C, Chhatwal A, Scott WE, et al. Transcriptional analysis identifies novel biomarkers associated with successful ex-vivo perfusion of human donor lungs. bioRxiv. 2019 Apr 17;612374.