

Scientific Letter

Histological Features and Gene Expression Profiling in Lung Transplantation From Donation After Circulatory Death



To the Director,

Recently, lung transplantation from donation after circulatory death (DCD) has become a routine clinical practice to address the donor shortage.^{1,2} However, lungs stored in warm conditions undergo more severe damage than those stored in cold conditions, and ischemia reperfusion injury (IRI) following warm ischemia (WIRI) is the most problematic issue in lung transplantation from DCD compared to donation from brain death donors (DBD). Although WIRI has been recognized as an enhancement of IRI following cold ischemia,³ recent studies highlight notable mechanistic differences.^{4–6} Furthermore, a comprehensive RNA sequencing analysis of lungs from a mouse WIRI model recently identified numerous genes expressed earlier than the transcription factor EGR-1, which has been considered the master regulator for pulmonary IRI.^{7–11} However, no specific drug exists to manage WIRI, emphasizing the need for further investigation. The objective of this study was to clarify the molecular mechanism of WIRI and identify therapeutic targets.

In this study, we analyzed human peripheral lung tissue biopsies collected 30 min after reperfusion. Five matched cases each of DCD and DBD lung transplantation were selected from a Spanish institutional database. The transplantations were performed between May 2020 and December 2020. As shown in [Supplemental Table 1](#), donor factors were matched for gender, age, and cause of death, and recipient factors were matched for gender, age, diagnosis, operative procedure, and perioperative outcome. H&E staining revealed that nuclear fragmentation was more pronounced in DCD lungs than in DBD lungs, indicating more evident DNA damage in DCD lungs ([Supplemental Fig. 1A](#)). This finding was supported by a TUNEL assay, which showed significantly higher TUNEL-positive cells in DCD lungs ($p = 0.0159$) ([Supplemental Fig. 1C](#)), suggesting differences in DNA damage status due to IRI between DCD and DBD lungs.

To understand the histological features of lung transplantations from DBD and DCD at molecular level, we conducted dynamic transcriptome analysis using RNA sequencing. Principal component analysis (PCA) demonstrated distinct RNA expression distributions between DBD and DCD lungs ([Fig. 1A](#)). Genes related to cell cycle and DNA damage and repair, such as PCNA, CCNA2, CDC25A, CDK1, and BRCA1, were more highly expressed in DCD lungs than in DBD lungs, while inflammation-related genes like MMP9, IFN- γ , IL-1 β , IL-6, and CXCL-8 were more prominent in DBD lungs ([Fig. 1B](#)). Canonical pathway analysis further examined pathways

associated with differentially expressed genes, revealing distinct pathways activated in DBD and DCD lungs ([Fig. 1C](#)). Specifically, genes associated with the Cell Cycle Control of Chromosomal Replication and Kinetochore Metaphase Signaling pathways were highly enriched in DCD lungs. [Supplemental Figs. 2 and 3](#) show signal maps with upregulated genes in these pathways. Conversely, TREM1 signaling and the role of IL-17F in allergic inflammatory airway diseases were significantly upregulated in DBD lungs, as visualized in [Supplemental Fig. 4](#). These findings underscore distinct gene expression profiles in DCD and DBD lungs, particularly in DNA damage response and inflammatory signaling, respectively.

Further upstream analysis using IPA (Ingenuity® Pathway Analysis) clarified the clustering of RNA-sequencing data, identifying FOXM1, CKAP2L, E2F1, and PCLAF as upstream signals activated in DCD lungs ([Supplemental Fig. 5](#)). These signals are closely related to DNA damage and replication. Immunohistochemistry with anti- γ H2AX antibody was used to quantify DNA damage at the protein level, with significantly more γ H2AX-positive nuclei in DCD than in DBD lungs ([Fig. 2](#)). γ H2AX-positive nuclei were significantly more in the DCD lungs than DBD lungs. γ H2AX is a critical indicator of cellular response to DNA damage, particularly double-strand breaks,^{12–14} contributing to DNA repair, cell cycle regulation, and programmed cell death. While γ H2AX is a sensitive DNA damage marker, no therapeutic agents targeting γ H2AX have been reported, including in organ transplantation or cancer.

To evaluate DNA replication levels in transplanted lungs, we quantified PCNA expression via immunofluorescence ([Fig. 2](#)). DCD lungs showed significantly more PCNA-positive cells than DBD lungs, indicating increased DNA replication. While cells routinely repair DNA damage caused by various stimuli, reactive oxygen species can induce DNA damage that activates poly ADP-ribose polymerase (PARP), which both repairs DNA and, under oxidative stress, promotes apoptosis and necrosis.¹⁵ Prior research by Hatachi et al. demonstrated that PARP inhibitors reduced inflammation and tissue damage in WIRI using a rat hilar clamp model.¹⁶

Despite limited studies on DNA repair targeting pulmonary IRI, Tan et al. reported that mitochondria-targeted administration of 8-oxoguanine DNA glycosylase-1 (OGG1), involved in DNA repair, suppressed IRI in a rat ex vivo model.¹⁷ They observed that OGG1 reduced mitochondrial DNA damage and decreased pro-inflammatory mitochondrial DNA fragments in lung perfusate. Ex vivo lung perfusion (EVLP) may offer a practical approach for DNA repair in DCD lungs. While stem cell, mesenchymal cell, and gene therapies are also potential treatments for DNA repair, no studies have demonstrated that these technologies improve transplanted lung function.

In DBD lungs, IFN- γ and IL-1 β signaling were identified as upstream pathways ([Supplemental Fig. 5](#)), with MMP9 and CXCL-

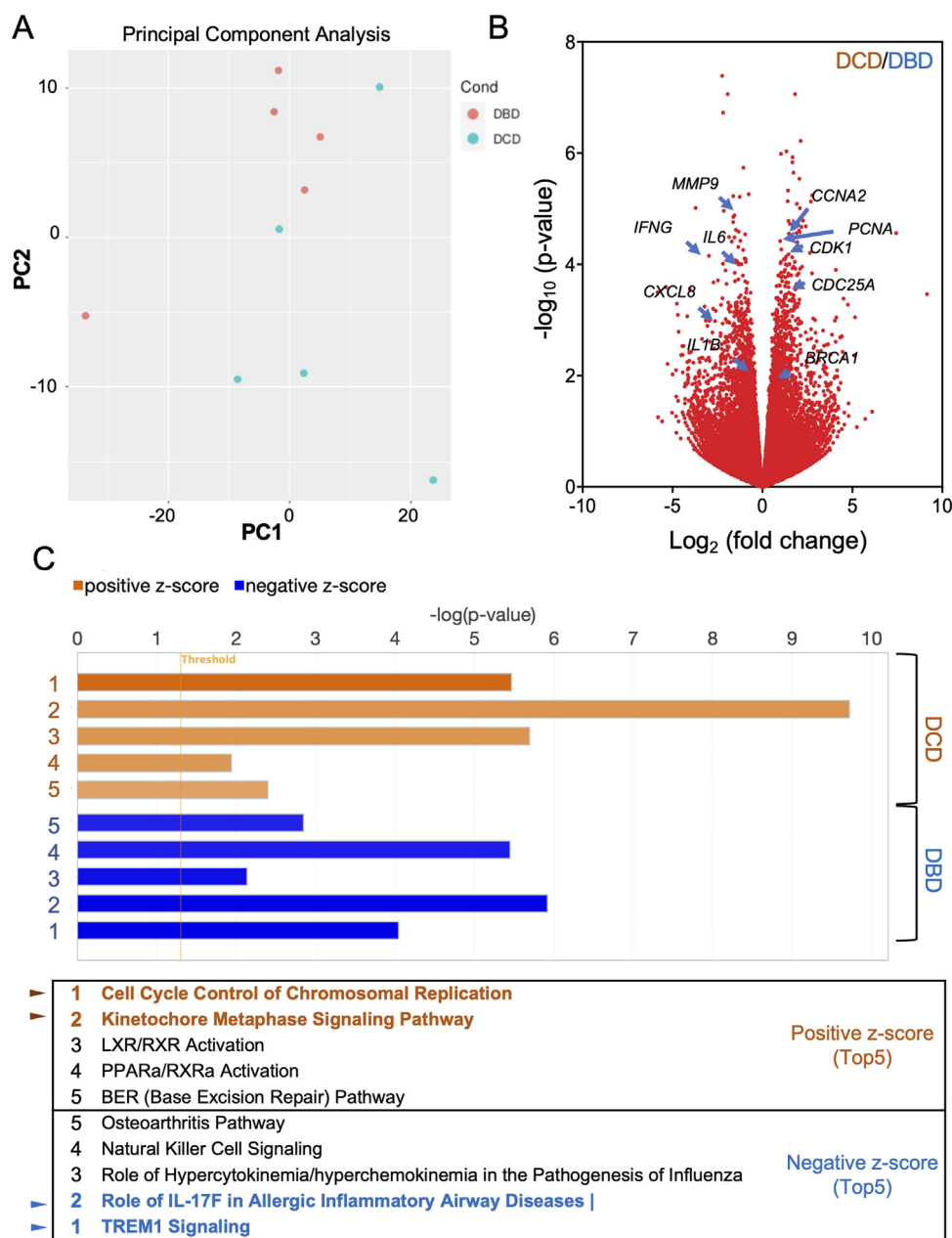


Fig. 1. Differences between lung transplantation from DBD and DCD by dynamic transcriptome analysis with RNA sequencing. (A) The distribution of RNA expressions in DBD and DCD lungs by principal component analysis. (B) Volcano plot represents genes highly expressed in each of DBD and DCD lungs. (C) Canonical pathway analysis represents pathways activated in DCD or DBD lungs. Orange: activation in DCD; blue: activation in DBD.

2 showing strong upregulation among inflammation-associated genes. Immunofluorescence analysis confirmed significantly more MMP9-positive cells in DBD than in DCD lungs (Fig. 2).

Disease and function analysis via IPA was conducted to investigate associations with previously published evidence (Supplemental Fig. 5). DCD lung had high homology to colorectal tumor and neoplasm, colorectal lesion, hereditary connective tissue disorder, and alignment of chromosomes. On the other hand, DBD lungs had high homology to the cell movement of phagocytes and myeloid cells, adhesion of immune cells, leukopoiesis, and recruitment of granulocytes. Cancer research has recently advanced therapeutic approaches targeting DNA damage responses,^{18,19} which may also be applied to DCD lung transplantation.

To our knowledge, this is the first study to analyze clinical DCD lung transplant specimens using RNA sequencing and immunofluorescence validation. Kang et al. previously employed microarrays

to reveal differences in gene expression profiles between DBD and DCD lungs pre-transplant, with a notable reduction in these differences post-transplant.⁴ Further, their recent study on pre-transplant DBD and DCD lungs identified distinct transcriptomic signatures: DBD lungs displayed elevated inflammatory cytokine expression, while DCD lungs had signatures linked to cell death, apoptosis, and necrosis.²⁰ While consistent with our findings, our study provides the first RNA-sequencing and immunofluorescence validation data for DCD lungs.

In conclusion, clinical lung tissues from DCD lung transplants exhibit significantly different gene expression profiles compared to DBD transplants. A combination of histological and transcriptomic analyses highlighted upregulation of DNA damage and replication signaling in DCD lungs, identifying DNA damage and replication as potential therapeutic targets for WIRI in DCD lung transplantation.

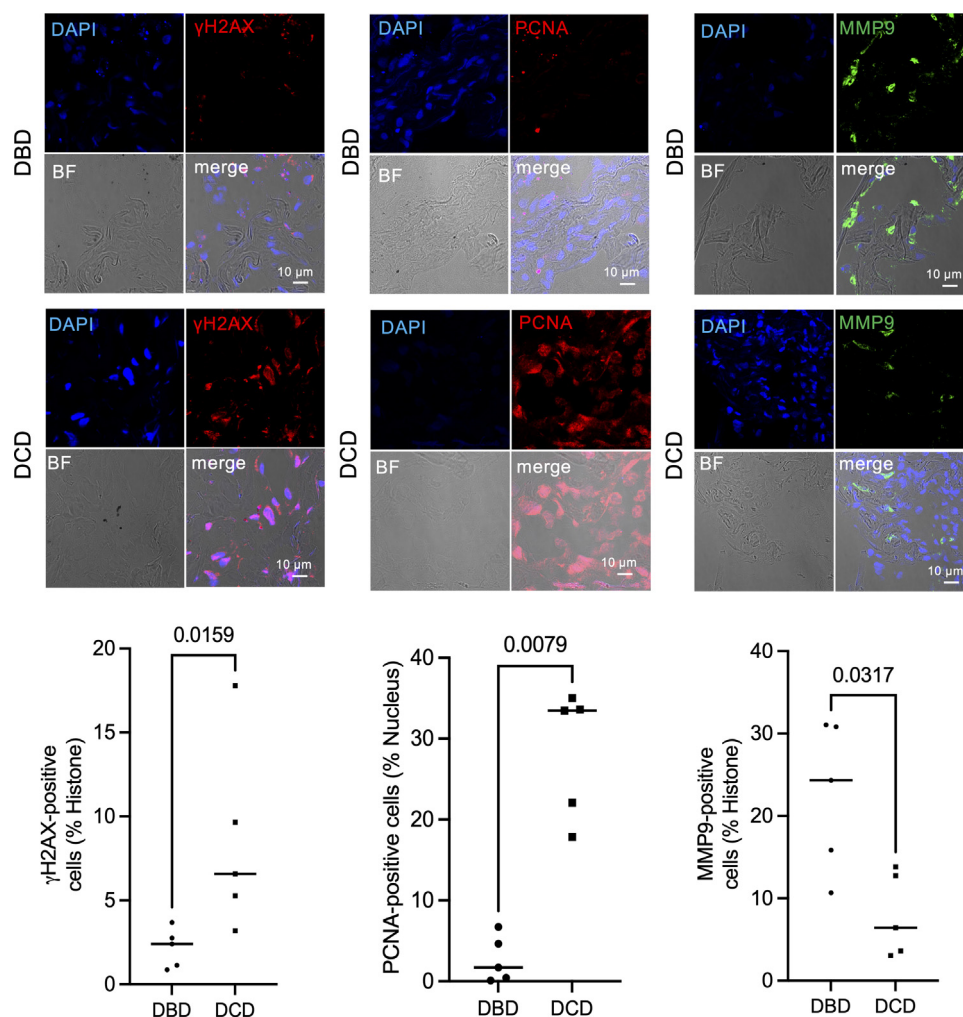


Fig. 2. Immunofluorescence staining of γ H2AX, PCNA, and MMP9 in DCD and DBD lungs. Representative immunofluorescence staining of γ H2AX, PCNA, and MMP9 in DCD and DBD lungs, and number of γ H2AX, PCNA, and MMP9 positive cells in DCD and DBD lungs. Data and bars are expressed as plots and medians ($n = 5$).

Author's Contributions

Concept and design: MO and TS; Collection of tissue samples: ST and YK; Analysis and interpretation of data: MO, TS and ST; Drafting/writing of the manuscript: MO, TS, ST; Manuscript reviewing: ST and DG; Technical support: ER, AS, MJC, LH and AR.

Ethics Statement

This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University Hospital, Okayama, Japan (approval number: 2105-039), Ehime University Hospital, Toon, Japan (approval number: 2406011), and Hospital Universitario Puerta de Hierro-Majadahonda (approval number: AD0021-A-2015).

Funding Statement

This research was funded by Grants-in-Aid for Scientific Research from Japanese Society for the Promotion of Science (20KK0203), and a research grant from the Strategic Action on Health 2021–2023 from Instituto de Salud Carlos III (dossier PI22-01592).

Conflict of Interests

The authors declare no competing interests.

Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.arbres.2024.12.015](https://doi.org/10.1016/j.arbres.2024.12.015).

References

- Ehram JP, Benden C, Immer FF, Inci I. Current status and further potential of lung donation after circulatory death. *Clin Transplant*. 2021;35:e14335, [http://dx.doi.org/10.1111/ctr.14335](https://doi.org/10.1111/ctr.14335).
- Furukawa M, Noda K, Chan EG, Ryan JP, Coster JN, Sanchez PG. Lung transplantation from donation after circulatory death, evolution, and current status in the United States. *Clin Transplant*. 2023;37:e14884, [http://dx.doi.org/10.1111/ctr.14884](https://doi.org/10.1111/ctr.14884).
- Warnecke G, Sommer SP, Gohrbandt B, Fischer S, Hohlfeld JM, Niedermeyer J, et al. Warm or cold ischemia in animal models of lung ischemia–reperfusion injury: is there a difference? *Thorac Cardiovasc Surg*. 2004;52:174–9, [http://dx.doi.org/10.1055/s-2004-817977](https://doi.org/10.1055/s-2004-817977).
- Kang CH, Anraku M, Cypel M, Sato M, Yeung J, Gharib SA, et al. Transcriptional signatures in donor lungs from donation after cardiac death vs after brain death: a functional pathway analysis. *J Heart Lung Transplant*. 2011;30:289–98, [http://dx.doi.org/10.1016/j.healun.2010.09.004](https://doi.org/10.1016/j.healun.2010.09.004).
- Yamamoto S, Okazaki M, Yamane M, Miyoshi K, Otani S, Kakishita T, et al. Peculiar mechanisms of graft recovery through anti-inflammatory responses after

- rat lung transplantation from donation after cardiac death. *Transpl Immunol*. 2012;26:133–9, <http://dx.doi.org/10.1016/j.trim.2011.11.002>.
6. Halloran K, Mackova M, Parkes MD, Hirji A, Weinkauff J, Timofte IL, et al. The molecular features of chronic lung allograft dysfunction in lung transplant airway mucosa. *J Heart Lung Transplant*. 2022;41:1689–99, <http://dx.doi.org/10.1016/j.healun.2022.08.014>.
 7. Yan SF, Lu J, Zou YS, Soh-Won J, Cohen DM, Buttrick PM, et al. Hypoxia-associated induction of early growth response-1 gene expression. *J Biol Chem*. 1999;274:15030–40, <http://dx.doi.org/10.1074/jbc.274.21.15030>.
 8. Yamamoto S, Yamane M, Yoshida O, Waki N, Okazaki M, Matsukawa A, et al. Early growth response-1 plays an important role in ischemia–reperfusion injury in lung transplants by regulating polymorphonuclear neutrophil infiltration. *Transplantation*. 2015, <http://dx.doi.org/10.1097/TP.0000000000000783>.
 9. Nakata K, Okazaki M, Sakaue T, Kinoshita R, Komoda Y, Shimizu D, et al. Functional blockage of S100A8/A9 ameliorates ischemia–reperfusion injury in the lung. *Bioengineering*. 2022;9:673.
 10. Okazaki M, Krupnick AS, Kornfeld CG, Lai JM, Ritter JH, Richardson SB, et al. A mouse model of orthotopic vascularized aerated lung transplantation. *Am J Transplant*. 2007;7:1672–9, <http://dx.doi.org/10.1111/j.1600-6143.2007.01819.x>.
 11. Kawana S, Okazaki M, Sakaue T, Hashimoto K, Nakata K, Choshi H, et al. Loss of Nr4a1 ameliorates endothelial cell injury and vascular leakage in lung transplantation from circulatory-death donor. *J Heart Lung Transplant*. 2024, <http://dx.doi.org/10.1016/j.healun.2024.09.028>.
 12. Kuo LJ, Yang LX. Gamma-H2AX – a novel biomarker for DNA double-strand breaks. *In Vivo*. 2008;22:305–9.
 13. Kinner A, Wu W, Staudt C, Iliakis G. Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res*. 2008;36:5678–94, <http://dx.doi.org/10.1093/nar/gkn550>.
 14. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, et al. GammaH2AX and cancer. *Nat Rev Cancer*. 2008;8:957–67, <http://dx.doi.org/10.1038/nrc2523>.
 15. Caldecott KW. DNA single-strand break repair. *Exp Cell Res*. 2014;329:2–8, <http://dx.doi.org/10.1016/j.yexcr.2014.08.027>.
 16. Hatachi G, Tsuchiya T, Miyazaki T, Matsumoto K, Yamasaki N, Okita N, et al. The poly(adenosine diphosphate-ribose) polymerase inhibitor PJ34 reduces pulmonary ischemia–reperfusion injury in rats. *Transplantation*. 2014;98:618–24, <http://dx.doi.org/10.1097/tp.0000000000000305>.
 17. Tan YB, Pastukh VM, Gorodnya OM, Mulekar MS, Simmons JD, Machuca TN, et al. Enhanced mitochondrial DNA repair resuscitates transplantable lungs donated after circulatory death. *J Surg Res*. 2020;245:273–80, <http://dx.doi.org/10.1016/j.jss.2019.07.057>.
 18. O'Connor MJ. Targeting the DNA damage response in cancer. *Mol Cell*. 2015;60:547–60, <http://dx.doi.org/10.1016/j.molcel.2015.10.040>.
 19. Huang R, Zhou PK. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduct Target Ther*. 2021;6:254, <http://dx.doi.org/10.1038/s41392-021-00648-7>.
 20. Baciuc C, Sage A, Zamel R, Shin J, Bai XH, Hough O, et al. Transcriptomic investigation reveals donor-specific gene signatures in human lung transplants. *Eur Respir J*. 2021;57, <http://dx.doi.org/10.1183/13993003.00327-2020>.
- Mikio Okazaki^{a,*}, Tomohisa Sakaue^{b,c}, Shin Tanaka^a, Yujiro Kubo^{d,e}, Tatsuya Hayashi^a, Elvira Ramil^f, Antonio J. Sánchez-López^{g,h}, María Jose Coronadoⁱ, Lucas Hoyos^{d,e}, Alejandra Romero^{d,e}, Shinichi Toyooka^a, David Gomez-de-Antonio^{d,e}
- ^a Department of General Thoracic Surgery and Breast and Endocrinological Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
^b Department of Cardiovascular and Thoracic Surgery, Ehime University Graduate School of Medicine, Ehime, Japan
^c Department of Cell Growth and Tumor Regulation, Proteo-Science Center (PROS), Ehime University, Ehime, Japan
^d Department of Thoracic Surgery and Lung Transplantation, Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain
^e Surgery Department, Medical School, Universidad Autónoma de Madrid (UAM), IDIPHISA, Madrid, Spain
^f Sequencing and Molecular Biology Unit, Hospital Universitario Puerta de Hierro-Majadahonda, IDIPHISA, Madrid, Spain
^g Biobank, Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana, Madrid, Spain
^h Neuroimmunology Unit, Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana, Madrid, Spain
ⁱ Confocal Microscopy Unit, Hospital Universitario Puerta de Hierro-Majadahonda, IDIPHISA, Madrid, Spain
- *Corresponding author.
 E-mail address: mikiookazaki@okayama-u.ac.jp (M. Okazaki).