



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Response to: Are endothelial cell proliferation and mesenchymal transition as distinguishing characteristics of three-week SuHx mice model?

Citation for published version:

Rodor, J, Chen, S-H & Baker, AH 2023, 'Response to: Are endothelial cell proliferation and mesenchymal transition as distinguishing characteristics of three-week SuHx mice model?', *Cardiovascular Research*.
<https://doi.org/10.1093/cvr/cvad075>

Digital Object Identifier (DOI):

[10.1093/cvr/cvad075](https://doi.org/10.1093/cvr/cvad075)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Cardiovascular Research

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



1 **Response to: Are endothelial cell proliferation and mesenchymal transition as distinguishing**
2 **characteristics of three-week SuHx mice model?**

3
4 Julie Rodor, Shiau-Haln Chen, Andrew H. Baker*

5
6 **Affiliations**

7 The Queen's Medical Research Institute, Centre for Cardiovascular Science, University of
8 Edinburgh, Edinburgh EH16 4TJ, UK.

9
10 * Corresponding author: Andrew H. Baker (Andy.Baker@ed.ac.uk; (0044) (0)131-242 6728)

11
12
13 We would like to thank the reader for their interest in our paper, and for their thoughts on
14 endothelial cell (EC) proliferation and endothelial-to-mesenchymal transition (EndMT) in the
15 Sugen5416/Hypoxia (SuHx) model of pulmonary arterial hypertension (PAH).

16
17 Our study reported no increase in proliferating ECs in SuHx PAH mice¹, which the reader
18 disagreed with. They reasoned this was because the differentially expressed genes between
19 control and PAH were associated with the gene ontology (GO) terms regulation of angiogenesis
20 (GO:0045765), regulation of vasculature development (GO:1901342), and EC proliferation
21 (GO:0001935). We believe that GO term analysis alone is insufficient for making conclusions
22 about cell proliferation. The GO terms highlighted by the reader also contain genes that regulate
23 those processes in either a positive or negative direction, or both. Importantly, angiogenesis
24 involves an intricate coordination and balance of EC proliferation, sprouting and migration. It is
25 overly simplistic to assume that because angiogenesis is induced, ECs must therefore be
26 proliferating. Various studies have also provided evidence showing that opposing EC
27 proliferative and migratory responses could present together in different pathologies and
28 mechanistic contexts²⁻⁴. The reader also suggested upregulation of *Bmpr2*, caveolin (*Cav*) 1/2,
29 and secreted protein acidic and cysteine rich (*Sparc*) in PAH as markers of EC proliferation. This
30 not only conflicts with our observation of *Bmpr2* downregulation in PAH¹, but also with the
31 expression plot in their letter and findings in the wider PAH field of reduced BMPR2 signalling⁵,
32 EC hyperproliferation in *Cav1* and *Cav2* knockout mice⁶, and unclear contributions of SPARC in
33 proliferation and angiogenesis⁷. Furthermore, we do not believe they are as direct or
34 established a marker of proliferation as cell cycle-related genes (e.g. *Mki67*, *Pcna*, *Mcm* genes).
35 We are thus confident in our finding, which is based on robustly assessing established
36 proliferation/cell cycle markers and the discrete cell cycle phases of each EC.

37
38 In their letter, the reader raised questions about our observation of limited EndMT in
39 control and SuHx-induced PAH, reporting very high levels of Acta2+ control ECs in their analysis
40 of murine whole lungs. We do not expect ECs to express any mesenchymal markers in control.
41 As such, the 24% of Acta2+ control ECs they reported seems unexpectedly high, unlike the 1%
42 Acta2+ control ECs we observed¹, though it is unclear from their plot label if it is 24% or 0.24%.
43 Their analysis also showed control and IH having similar proportions of Acta2+ ECs. This conflicts

© The Author(s) 2023. Published by Oxford University Press on behalf of the European Society of Cardiology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

1 with their claim and matches our finding, which they had disputed. Although the reader
2 provided immunofluorescent staining of PECAM1 and ACTA2 in lung cryosections from mice in
3 Normoxia or SuHx, there was lack of quantification of double-positive cells and statistical
4 evaluation of the difference between groups. Furthermore, they have only evaluated one
5 EndMT marker, ACTA2, where we know that a suite of markers should be used⁸. EndMT is in
6 itself a highly complex phenomenon and field of study, and Kovacic *et al.*⁸ has extensively
7 reviewed the challenges posed by a lack of standard molecular definition. We used a panel of
8 genes that could mark the following EndMT characteristics: reduced endothelial phenotype,
9 increased mesenchymal phenotype, and increased expression of EndMT mediators^{1,8}. Our
10 comprehensive assessment did not reveal any EC populations distinctly expressing these
11 markers, suggesting EndMT was not present in our data. We also have no reason to believe that
12 the tamoxifen induction efficiency and the fluorescence activated cell sorting (FACS) strategy we
13 used are limitations in this context. EndMT has previously been studied in tamoxifen-induced
14 condition⁹. We found 95.5% of *TdTomato*^{high} cells in our *Cdh5-CreERT2-TdTomato* mouse line,
15 which were also *Cdh5*^{high} and *Pecam1*^{high} and were independently annotated as ECs by *SingleR*¹.
16 Considering how EndMT could present as a transient phenomenon⁹ and how scRNA-seq only
17 reveals a static snapshot of the cell state, we acknowledge that it is possible for EndMT to have
18 occurred in earlier stages of our model, but this was not captured in our study. Importantly, we
19 would like to emphasise that EndMT in PAH, and the wider cardiovascular disease field, has thus
20 far been largely observational (i.e. being associated with a disease) and not causal⁸.

21
22 The final point raised by the reader was that our functional validation of CD74 should
23 have focused on its immune function, rather than its impact on EC proliferation and barrier
24 integrity. They also proposed using pulmonary artery ECs (PAECs), instead of human umbilical
25 vein ECs (HUVECs). PAH is a complex disease that involves not only an immune component, and
26 the immune contribution of CD74 in ECs has been previously studied¹⁰. However, the exact
27 contribution of CD74 to the non-immune components of EC dysfunction in PAH remains unclear.
28 We thus chose to extend our investigation of *CD74* beyond inflammation, through targeted
29 assays of EC proliferation and barrier function, with HUVECs as an initial *in vitro* model system,
30 before extending these findings further into other EC types and ultimately into *in vivo* models.

31
32 Conflict of interest: none declared.

33 34 **References**

- 35
36 1. Rodor, J. *et al.* Single-cell RNA-seq profiling of mouse endothelial cells in response to
37 pulmonary arterial hypertension. *Cardiovasc. Res.* (2021) doi:10.1093/cvr/cvab296.
- 38 2. McDonald, A. I. *et al.* Endothelial Regeneration of Large Vessels Is a Biphasic Process
39 Driven by Local Cells with Distinct Proliferative Capacities. *Cell Stem Cell* **23**, 210-225.e6
40 (2018).
- 41 3. Li, Z. *et al.* Single-cell transcriptome analyses reveal novel targets modulating cardiac
42 neovascularization by resident endothelial cells following myocardial infarction. **40**,
43 2507–2520 (2019).
- 44 4. Pontes-Quero, S. *et al.* High mitogenic stimulation arrests angiogenesis. *Nat. Commun.*

- 1 2019 101 **10**, 1–16 (2019).
- 2 5. Evans, J. D. W. *et al.* BMPR2 mutations and survival in pulmonary arterial hypertension:
3 an individual participant data meta-analysis. *Lancet. Respir. Med.* **4**, 129 (2016).
- 4 6. Razani, B. *et al.* Caveolin-1 Null Mice Are Viable but Show Evidence of Hyperproliferative
5 and Vascular Abnormalities. *J. Biol. Chem.* **276**, 38121–38138 (2001).
- 6 7. Rivera, L. B., Bradshaw, A. D. & Brekken, R. A. *The regulatory function of SPARC in vascular*
7 *biology. Cellular and Molecular Life Sciences* vol. 68 3165–3173 (Cell Mol Life Sci, 2011).
- 8 8. Kovacic, J. C. *et al.* Endothelial to Mesenchymal Transition in Cardiovascular Disease: JACC
9 State-of-the-Art Review. *J. Am. Coll. Cardiol.* (2019) doi:10.1016/j.jacc.2018.09.089.
- 10 9. Tombor, L. S. *et al.* Single cell sequencing reveals endothelial plasticity with transient
11 mesenchymal activation after myocardial infarction. *Nat. Commun.* **2021 121 12**, 1–12
12 (2021).
- 13 10. Le Hiress, M. *et al.* Proinflammatory Signature of the Dysfunctional Endothelium in
14 Pulmonary Hypertension. Role of the Macrophage Migration Inhibitory Factor/CD74
15 Complex. *Am. J. Respir. Crit. Care Med.* **192**, 983–997 (2015).
- 16
17