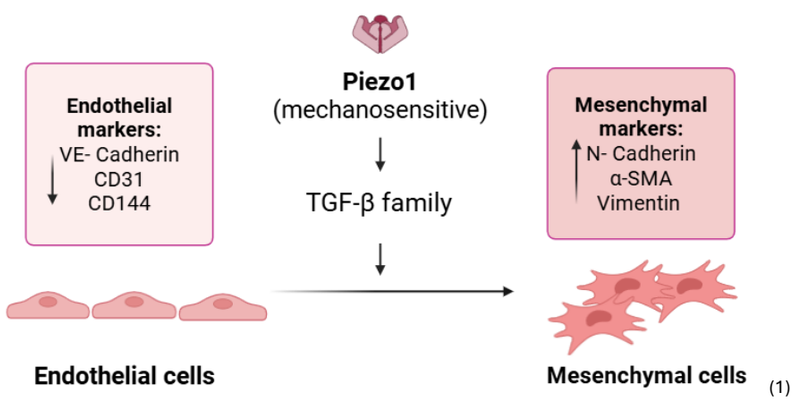
Pilot investigation of PIEZO1 in endothelial to mesenchymal transition (EndMT) of human umbilical vein endothelial cells

James Pasint-Magyar[1,2], Sophie Earle[1,2], Georgia Fakonti [2], Tudor Dimofte[2], Nigel Simpson[1,2], David Beech[2] , Lara Morley[1,2]

1. Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK
2. Leeds Teaching Hospitals NHS Trust

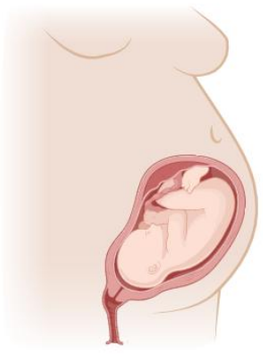
Background

1)



(1)

2) Fetal growth restriction (FGR) is associated with vascular fibrosis in the placenta.[(2)] Understanding the pathways of fibrosis in the placenta is key to uncovering novel therapeutics.PIEZO1 is known to be expressed by placental endothelial cells.[(3)]



3) This pilot study aimed to explore the role of PIEZO1 in EndMT in human umbilical vein endothelial cells (HUVECs) under static and shear conditions and develop an experimental protocol for EndMT induction.

Methods

Samples and cell-culture

Three biological repeats of cultured HUVECs were were transfected with either 50nmol non-targeting control siRNA or PIEZO1 target-specific siRNA.

EndMT induction

48 hours after transfection, HUVECs were treated with TGF-β2 and IL-1β (10ng/ml) for a further 48 hours under static or shear conditions. HUVECs were also treated with dimethyl sulphoxide control or 0.2 μM Yoda1 (PIEZO1 agonist).

Flow cytometry

HUVECs were stained with PE-CD144 (B4 peak channel), an endothelial marker and APC-CD325 (V1 peak channel), a mesenchymal marker. Flow cytometric analysis was performed on a 3 Laser Cytek Aurora Evo instrument.

Immunocytochemistry

HUVECs were fixed with 4% PFA, permeabilised and blocked. Cells were incubated with primary antibodies to endothelial markers: PECAM1 (CD31),CD144 and mesenchymal markers: alpha smooth muscle actin (α-SMA) and vimentin followed by Alexa Fluor 488 and 647. Images were analysed with QuPath (v 0.7.0)

Western blotting

Extracted protein lysates underwent gel-electrophoresis and membrane transfer. Membranes were incubated with anti-PIEZO1 or antibody and imaged with iBright™ imaging.

Statistical analysis

Statistical analysis included t-test, Mann-Whitney U and Kruskal-Wallis. Analysis was performed using Prism (v11.0.0).

Results

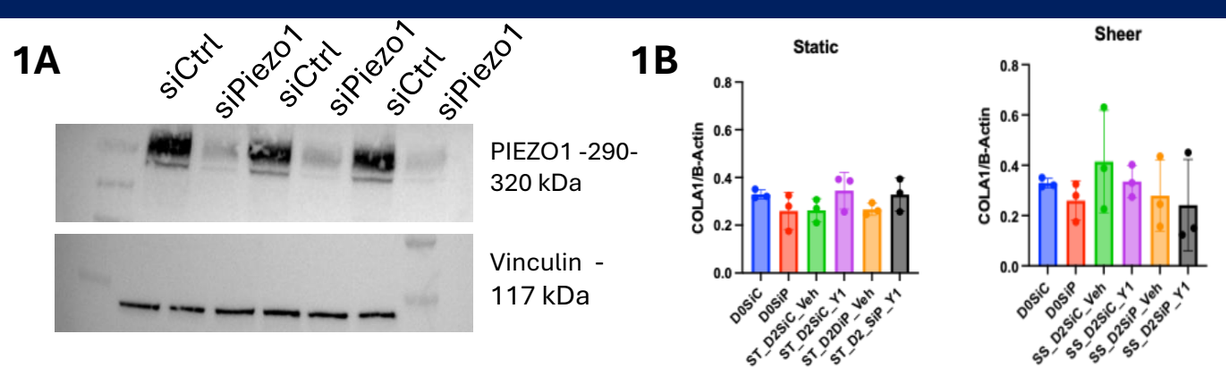


Figure 1. 1A. PIEZO1 expression is significantly reduced with PIEZO1 knock-down after transfection with siRNA (SiCtrl Vs SiPiezo1) demonstrated via Western Blotting on HUVECs ($p<0.001$). Loading control vinculin. **1B. No difference in mesenchymal Collagen1 expression across all conditions** from day 0 to day 2, demonstrated via Western blotting, n=3.

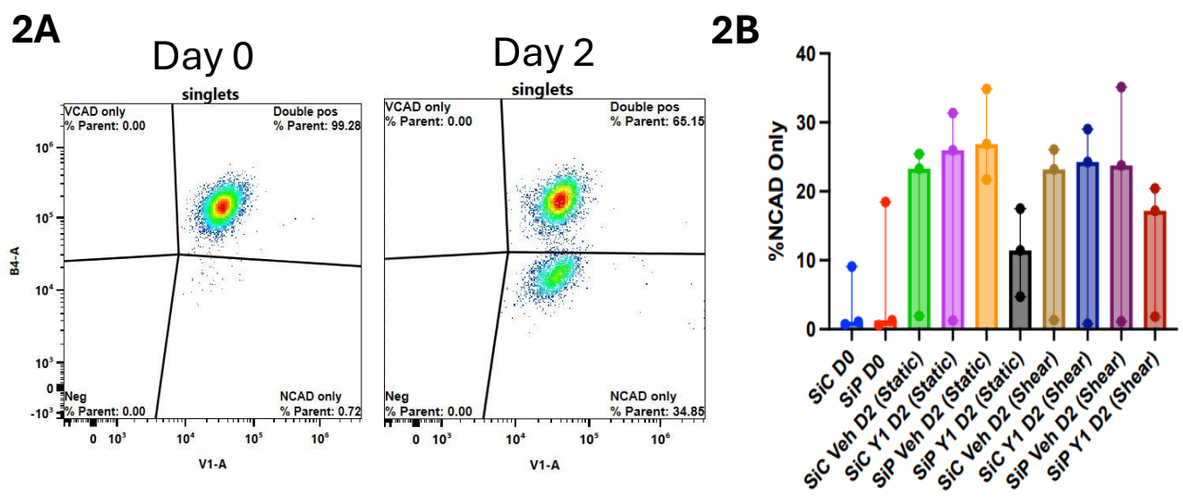


Figure 2. Figure 2A is a representative of increased %NCAD from day 0 to day 2 after Piezo1 knockdown from one of the HUVECs. Figure 2B is a bar chart showing %NCAD only expressing HUVECs, showing a **potential increase in mesenchymal NCAD between day 0 and day 2,** (n=3).

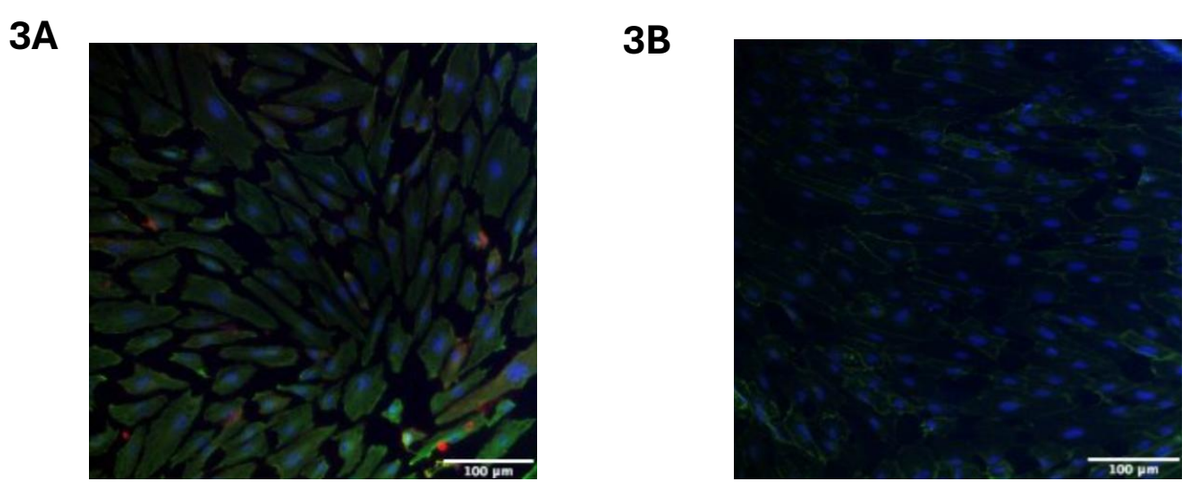


Figure 3. No differences in endothelial or mesenchymal expression after 2 days. Figure 3A is a representative immunocytochemistry image of dual stain for CD31 (red) and Vimentin (green) . Figure 3B is a representative image for CD144 (green) and α-SMA (red).

Western blotting

PIEZO1 protein expression was significantly reduced following knock-down ($p<0.001$) . PIEZO1 was detected at the expected molecular weight of 290-320 kDa (figure 1).

Flow cytometry

At D0 there was >90% double positive VE-Cadherin and N-Cadherin expression in the parent population (figure 2A). A potential increase in %N-cadherin only expressing HUVECs was seen between D0 and D2 (figure 2B).

Immunocytochemistry

No significant differences in expression were seen for CD144, CD31, Vimentin or α-SMA between D0 and D2 or across treatment groups. Representative images are seen in figure 3A and 3B.

Conclusions

Whilst no significant differences in mesenchymal markers were seen between day 0 and day 2 after EndMT induction, an increased trend in %NCAD only was observed, likely limited by sample size and variance. Data did not support regulation of EndMT by PIEZO1. However, experimental protocol optimisation is first required to further investigate the potential relationship between PIEZO1 and EndMT in the placenta and how this may relate to FGR.

References

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