The role of Piezo1 in blood-retinal barrier dysfunction

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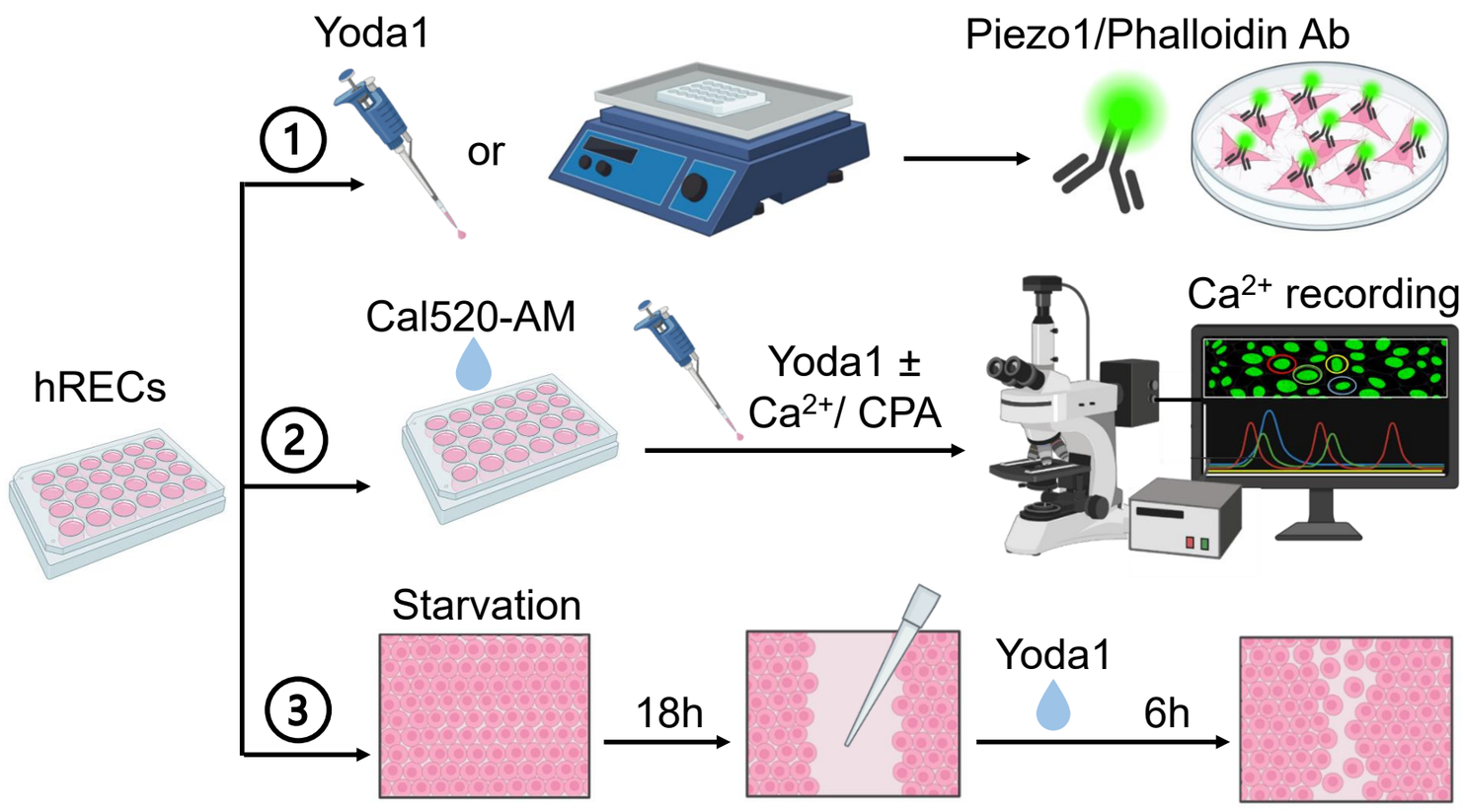
Introduction

- Many eye disorders stem from defects in the blood vessels of the retina that form the inner blood-retinal barrier (iBRB)[1].
- The iBRB is made of endothelial cells (ECs), which in pathological conditions become leaky and proliferate uncontrollably, mainly in response to the vascular endothelial growth factor (VEGFA)[2].
- Anti-VEGFs are the main therapy for retinopathies, but they work only on 50% of the patients, indicating that new therapies are urgently needed[3].
- Disturbances in blood flow characterise the early clinical stages of retinal disorders and cause increased shear stress and blood vessel damage[4].
- To uncover the role of vascular mechanotransduction in retinal physiology and disease, we investigated the mechanosensitive channel Piezo1[5,6].

Hypothesis

Piezo1 plays a relevant role in human retinal endothelial cell (hREC) physiology and VEGF signalling

Methods



Human retinal endothelial cells (hRECs) were seeded on collagen I-coated multiwell plates or glass coverslips and maintained in EGM-2 medium at 37ºC with 5% CO_2 until confluent.

1) hRECs were left untreated, treated with 1uM Yoda1 for 5 minutes or left on a shaker for 5 minutes before being fixed with 4% PFA. Cells were stained for Piezo1 or Phalloidin (green) against DAPI (blue).

2) hRECs were loaded with Cal520-AM for 1 h at 37ºC and washed with Krebs for 30 minutes. Fluorescence was recorded continuously before and after treatment.

3) hRECs were starved overnight. A wound was made with a pipette tip, and treatment was added for 6 h. Pictures were taken at 0 h and 6 h.

Results

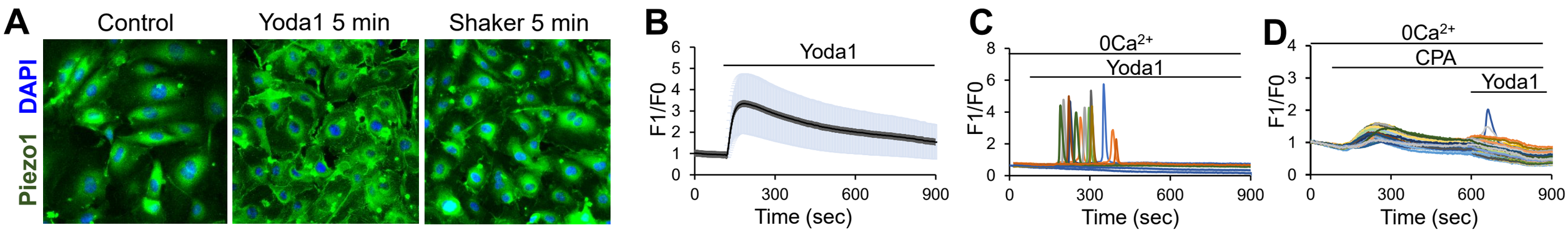


Figure1. Piezo1 is expressed and active in hRECs. (A) Piezo was broadly localised in untreated cells, whereas it migrated near the plasma membrane and perinuclear envelope after treatment. **(B-D)** Treatment with 1μM Yoda generated a single transient followed by a plateau in all the cells **(B)**. Around 20% of cells showed a short single transient in the absence of extracellular Ca^{2+} **(C)**, whereas 5% of cells responded to Yoda1 after passive depletion of the endoplasmic reticulum **(D)**.

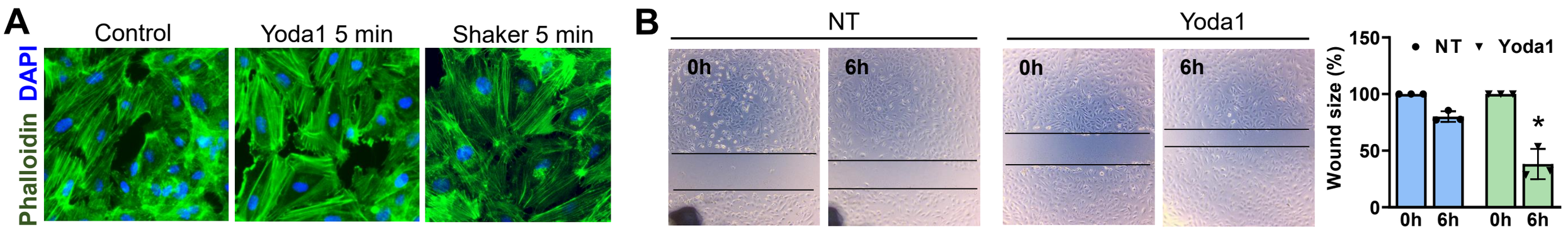


Figure 2. Piezo1 induces cytoskeletal changes and migration. (A) In untreated hRECs, the cytoskeletal actin was evenly distributed around the cells, whereas after treatment, it became more localised toward the plasma membrane and away from the nucleus. **(B)** Treatment with 1μM Yoda for 6 h led to an increase in the migration rate compared to the control. *p<0.05.

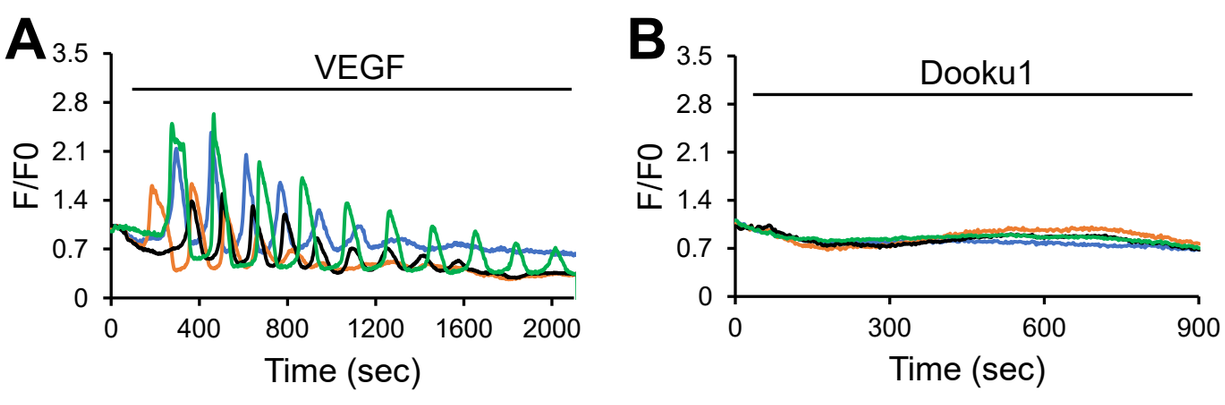


Figure 3. Piezo1 contributes to VEGF signalling. (A) Treatment of hRECs with VEGF (10 ng/ml) induced Ca^{2+} oscillations, which were prevented by 15 minutes of pre-treatment with Piezo1 inhibitor Dooku1 (1 μM) **(B-C)**.

Conclusions

Piezo1 plays a role in hREC functions

- Piezo1 is expressed and active in hRECs, and its location changes upon activation.
- Piezo1 activation leads to actin changes and cell migration.
- Inhibition of Piezo1 affects hREC response to VEGF.

References: 1) Hang et al., Int Ophthalmol. 2025. **2)** O'Leary and Campbell. FASEB J. 2023. **3)** Wallsh and Gallemore. Cells. 2021. **4)** Pournaras et al., Prog. Retin. Eye Res. 2008. **5)** Li et al., Nature. 2014. **6)** Harraz, et al. Circ Res. 2022.

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