PRIMARY CILIA-MEDIATED NITRIC OXIDE PRODUCTION IN SCHLEMM'S CANAL CELLS

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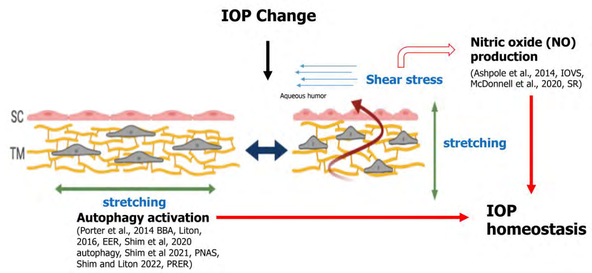
BACKGROUND

Intraocular pressure (IOP) homeostasis: Changes in IOP are "sensed" by stretching of trabecular meshwork (TM) cells and shear stress on Schlemm's canal (SC) cells in conventional outflow pathway.

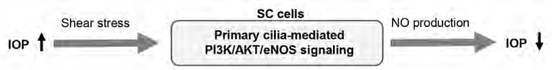


Primary cilium (PC) is a flow sensor: PC is a non-motile cell surface projection. Especially, the PC membrane is a specialized domain extension of the plasma membrane enriched on signaling receptors and channels, which enables the PC to function as a signaling hub. PC has been reported to act as a flow sensor and mechanotransductor in vascular endothelial and kidney epithelial cells (Anvarian et al., 2019, NRN; Saternos et al, 2018, NO; Luu et al, 2018, Atherosclerosis).

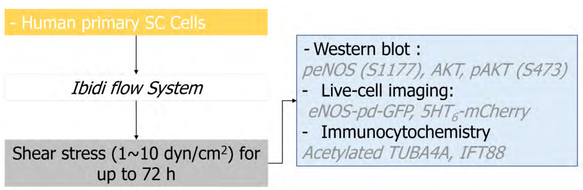
Is PC a mechanosensor in SC cells? it has not yet been evaluated whether PC are present and nitric oxide production is regulated by PC in response to shear stress in SC cells.

OBJECTIVES

- **Objective:** To identify the molecular mechanism by which regulates endogenous NO production in SC cells.
- **Hypothesis:** Primary cilium (PC), a non-motile cell surface projection, recognizes the elevated IOP-induced shear stress in SC cells, which induces NO production via PI3K/AKT/eNOS signaling pathway to reduce IOP.



EXPERIMENTAL OUTLINE



RESULTS

1 Optimization of long-term timelapse live cell imaging system during shear stress application

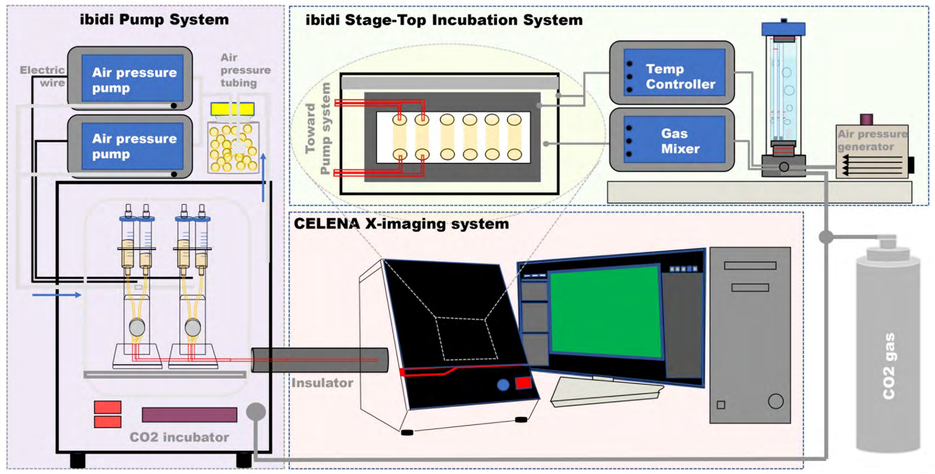


Fig.1. Schematic diagram for long-term timelapse live cell imaging system during shear stress application.

2 PC are present on SC cells and respond to shear stress

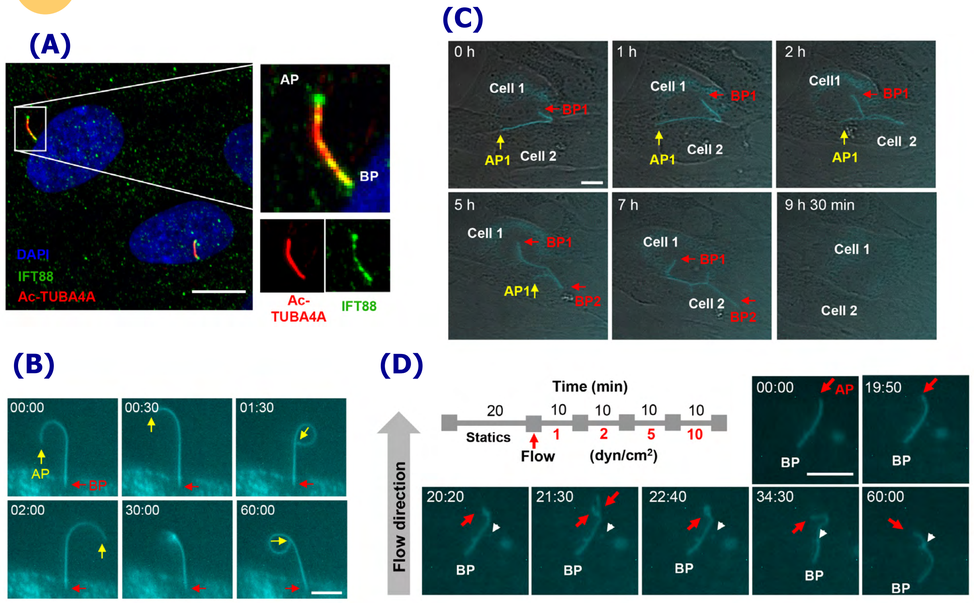


Fig. 2. PC are present and respond to shear stress in SC cells. (A) Representative immunocytochemical analysis of PC in SC cells. Acetylated TUBA4A (red fluorescence) and IFT88 (green fluorescence) antibodies were used to identify the PC. DAPI was used to stain nuclei. Images were acquired with confocal microscope and processed by using Fiji software. BP and AP represent basal and apical process, respectively. (B) Time-lapse live cell imaging of PC in SC cells transfected with p5HT6–mCherry. Images were acquired with CELENA® X live cell imaging system and processed by using Fiji software. BP and AP are indicated by red and yellow arrows, respectively. Captured time (min: sec) is represented in upper left of each picture. (C) Live cell imaging of PC dynamics. Note that the two cells are connected by forming PC network and the PC is disappeared in certain cell stages. (D) Response of PC upon fluid flow. AP is indicated by red arrows. Arrowheads represent a vesicle containing 5HT6–mCherry. Captured time (min: sec) is represented in upper left of each picture. Scale bars: 10 μm.

3 PC regulate shear stress-induced eNOS activation in SC cells

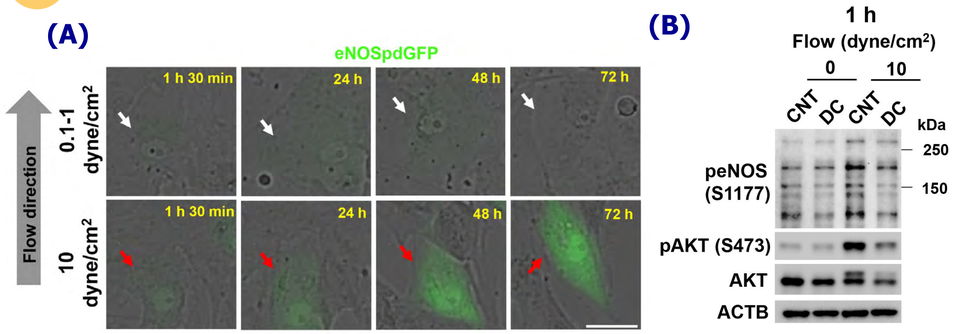


Fig. 3. PC regulate shear stress-induced eNOS activation. (A) Human SC cells were transduced by adenovirus expressing GFP under the control of eNOS promoter (eNOSpd-GFP) and the temporal regulation of eNOS expression were measured upon shear stress in SC cells. Scale bars:50 μm. (B) Western blot analysis for detecting peNOS and pAKT in deciliated human SC cells. CNT: control, DC: deciliated.

4 PI3K regulates shear stress-induced AKT activation in SC cells

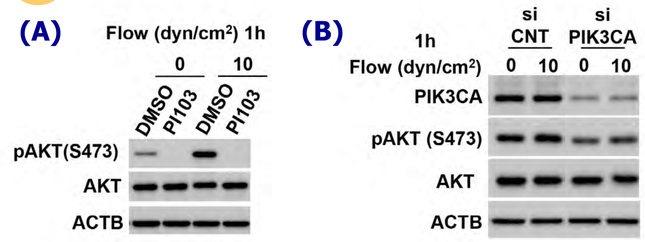


Fig. 4. PI3K inhibition prevents shear stress-induced AKT activation. PI3K is inhibited by chemically (PI103; A) or genetically using siRNA against PIK3C, a class I PI3K catalytic subunit (B).

CONCLUSIONS

In this studies, we have made significant progress:

1) Developing an optimized monitoring system that can measure NO production and dynamics in SC cells under shear stress conditions.
2) Demonstrating that PC regulates endothelial nitric oxide synthase in SC cells.
3) Additionally, our findings indicate that the phosphoinositide 3-kinase pathway is critical for eNOS activation in response to shear stress in SC cells.

NEXT STEP

We are currently working on and will continue:

1) Investigating the effect of disrupting PC and inhibiting PI3K/AKT signaling pathway on NO production directly in response to shear stress in SC cells.
2) Generating mice with disrupted PC in SC cells to investigate the effects of this disruption on IOP regulation.

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