Supplemental Material

Cold Exposure Reveals Two Populations of Microtubules in Pulmonary Endothelia

Cristhiaan D. Ochoa [1, 4], Troy Stevens[1, 2, 4], and Ron Balczon [3, 4]

Departments of Pharmacology[1]; Medicine[2]; Cell Biology and Neuroscience[3]; and Center for Lung Biology[4]; University of South Alabama, Mobile, AL 36688

Corresponding author: Ron Balczon, Ph.D.

Associate Professor

Department of Cell Biology and Neuroscience

Center for Lung Biology

College of Medicine

University of South Alabama

Mobile, AL 36688

Phone: (251) 460-6776

Fax: (251) 460-6771

rbalczon@usouthal.edu

Running Head: Cold-stability of Lung Endothelial Microtubules

Material and Methods:

Cell Culture: Pulmonary microvascular endothelial cells (internal identification: PMVECR1); were obtained from the Cell Culture Core at the University of South Alabama Center for Lung Biology and cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% heat-inactivated fetal bovine serum (Cat No. 10082; Invitrogen – Carslbad, CA) and 1% penicillin/streptomycin (Cat No. 15140; Invitrogen – Carslbad, CA).

Paclitaxel treatment: PMVECR1 were treated with Paclitaxel at 100nM for 3h as described earlier by our group (1).

Microtubule extraction protocol: After cold exposure, cells were rinsed with PBS and the buffer was removed. Next, 100 uL of PEM buffer containing 0.5% Triton X-100 and 100nM Paclitaxel was added to each dish to permeabilize the cells and release tubulin monomers (soluble tubulin). After 3 minutes, the extraction buffer was removed and the cells were rinsed with an additional 50 uL of buffer. The rinse buffer was collected and pooled with the initial extraction solution, and then 150 mL of 2x radio-immuno precipitation assay (RIPA) (Cat No. BP-115; Boston Bioproducts – Worcester, MA) with 1:100 protease inhibitors cocktail (Cat No. P8340; Sigma-Aldrich – St Louis, MO) was added to the pooled solution containing the soluble monomeric tubulin for a total volume of ~300uL. Next, cells ghosts (polymerized tubulin) were lysed with 300 uL of 1x RIPA buffer and protease inhibitor. Soluble and polymerized tubulin samples were stored at -80°C for future analyses.

Results:

Western blot analyses confirm cold-stable microtubules in endothelial cells. Western blot to detect α-tubulin reveals that at 37°C, endothelial cells have more polymerized than soluble tubulin (Figure S1A). Using this extraction protocol, cold-treatment did not appear to disassemble endothelial cell microtubules since no α-tubulin was detected in the soluble fraction. This finding confirms that endothelial has cold-stable microtubules. In HeLa cells, we were able to detect α-tubulin in the soluble fraction (Figure 1SB).

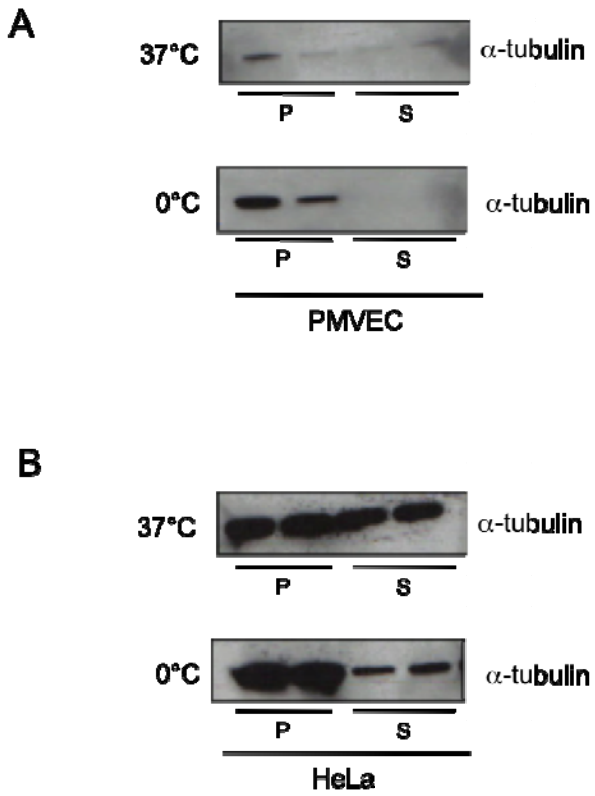
Discussion:

These findings should not be interpreted as to mean that all endothelial cell microtubules are cold-stable since we have documented in the main manuscript that there is in fact a subpopulation of microtubules that disassembles with cold. We believe that the brief Paclitaxel exposure was enough to reassemble depolymerized microtubules. This did not occur to the same degree in HeLa cells were the fraction of microtubules disassembled by cold is much larger.

Figure Legends:

Figure S1: Microtubules and α–tubulin in microvascular endothelium vs. HeLa cells. [S1A] At 37°C, PMVECs have more polymerized than soluble tubulin. *Upper panel.* Western blot analysis to detect α–tubulin in PMVEC at 37°C. *Lower panel*. Following cold exposure, PMVEC have no detectable amount of soluble tubulin. [S1B] At 37°C, HeLa cells have more polymerized than soluble tubulin. *Upper panel.* Western blot analysis to detect α–tubulin in HeLa cells at 37°C. *Lower panel*. Following cold exposure, HeLa cells have a detectable amount of soluble tubulin.

Figure S1



References

1. **Prasain N, Alexeyev M, Balczon R and Stevens T.** Soluble adenylyl cyclase-dependent microtubule disassembly reveals a novel mechanism of endothelial cell retraction. *Am.J.Physiol.Lung Cell.Mol.Physiol.* 297: 1: L73-83, 2009.