Online Data Supplement

Septin-2 mediates airway epithelial barrier function in physiologic and pathologic conditions.

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Materials and Methods

Membrane preparations. Samples were homogenized in cell lysis buffer (7.7mM Na phosphate buffer, 1mM NaN3, 1mM EDTA, 0.25 M sucrose, protease inhibitor cocktail (Sigma), phosphatase inhibitor cocktail (Sigma)) and the homogenate was centrifuged at 1000g for 10 min to discard the nonhomogenized material. Subsequently, the supernatant was centrifuged using an ultracentrifuge at 200,000g for 45 min. The pellet is enriched for membranes and is resuspended in a 5% SDS buffer for immunoblotting.

Fluorescence resonance energy transfer with acceptor photobleaching (FRET AB) was performed on fixed samples. The donor fluorescence intensity in a region of interest before and after acceptor photobleaching was compared. Positive FRET occurred if acceptor photobleaching resulted in increased donor fluorescence. The energy transfer efficiency EFRET is quantified by:
EFRET = (Dpost - Dpre)/Dpost = 1 - Dpre/Dpost
where
EFRET - Energy transfer efficiency;
Dpre - Donor fluorescence intensity before acceptor photobleaching**.**
Dpost - Donor fluorescence intensity after acceptor photobleaching;

Shear stress. We calculated the rate of perfusate flow in an open plate apparatus required to create shear stress comparable shear *in vivo*, assuming the perfusate is a Newtonian, non-compressible fluid with no-slip boundary conditions. Fluid flow rates of 0.5- 1ml/min provide a shear stress consistent with the magnitude for airway epithelial cells *in vivo*. Cells on inserts were placed on coverslips and perfused in a chamber (RH-2; Warner Instrument) at 0.5-1 ml/min with a Krebs solution. The perfusate was gassed with 16%O_2, 5% CO_2 at 37^{o}C. Chamber temperature was maintained with an in-line heat exchanger and heated platforms controlled by a dual channel servo controller (SF-28 and TC-344B, Warner Instrument). The cells were exposed to laminar shear stress (τ) calculated by: $\tau = 6\mu Q/wh2$, where Q= the flow, at 0.5-1 ml/min. The chamber dimensions (w,h) are 12.5 mm and 1mm; assuming that Krebs solution has a similar viscosity(μ) to water at (temperature 37^{o}C) μ = 0.00653 dyne*s/cm^2, then shear stress is τ= 1.5-3 dynes/cm2.