p38 MAPK mediates calcium oxalate crystal-induced tight junction disruption in distal renal tubular epithelial cells

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SUPPLEMENTARY METHODS

Cell cultivation and polarization

Madin-Darby canine kidney (MDCK) cells were cultivated in Eagle's minimum essential medium (GIBCO; Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum, 1.2% penicillin G/streptomycin and 2 mM L-glutamine. The cells were maintained in a humidified incubator at 37°C with 5% CO_2. MDCK cells were conditioned to be the polarized cells using Transwells^{TM} (Costar; Cambridge, MA). Briefly, the permeable polycarbonate membrane (0.4 μm pore size) was precoated with 6 μg/cm^2 collagen type IV (Sigma; St Louis, MO) dissolved in 0.25% acetic acid and incubated at 4°C overnight. Thereafter, MDCK cells (approximately 5.0-7.5×10^5 cell/ml) were plated and grown on the precoated permeable polycarbonate membrane insert for approximately 4 days, and the culture medium was refreshed every other day.

Isolation of apical and basolateral membranes

Briefly, the polarized MDCK cells were rinsed twice with ice-cold membrane preserving buffer (1 mM $MgCl_2$ and 0.1 mM $CaCl_2$ in PBS). Whatman filter paper (0.18-mm-thick, Whatman International Ltd.; Maidstone, UK), which was prewetted with deionized water, was placed onto the polarized cell monolayer for 5-min. Thereafter, the filter paper was peeled out and the apical membranes retained on filter paper were harvested by rehydration in deionized water. The remaining cell shafts were vigorously washed with PBS several times to remove cytoplasmic proteins, whereas the basolateral membranes were then harvested and resuspended in deionized water. Apical and basolateral membranes resuspended in deionized water were isolated by lyophilization.