Effects of inhibition of claudin-2, ZO-1, and occludin expression on paracellular permeability in HK-2 cells

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Introduction and Aims

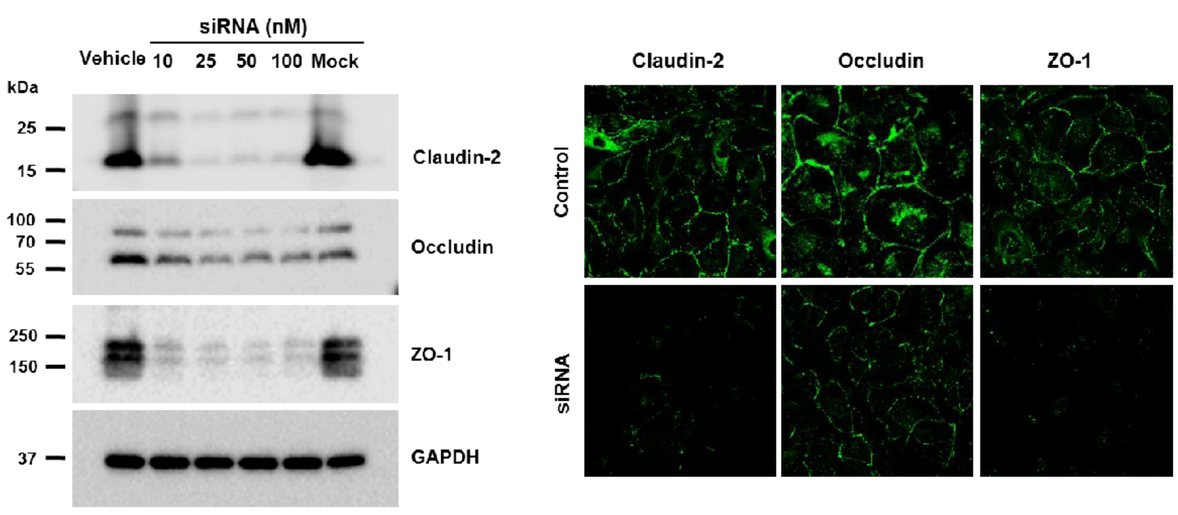
Claudin-2, ZO-1, and occludin are the major components of tight junctions (TJ) in the proximal tubule. However, their roles in maintaining paracellular permeability as leaky epithelia have yet to be defined. To investigate the role of TJ proteins in the leaky proximal tubule, we examined the effects of inhibition of claudin-2, ZO-1, and occludin expression on the transepithelial electrical resistance (TER) and paracellular permeability using the immortalized human proximal tubule epithelial cell line HK-2.

Methods

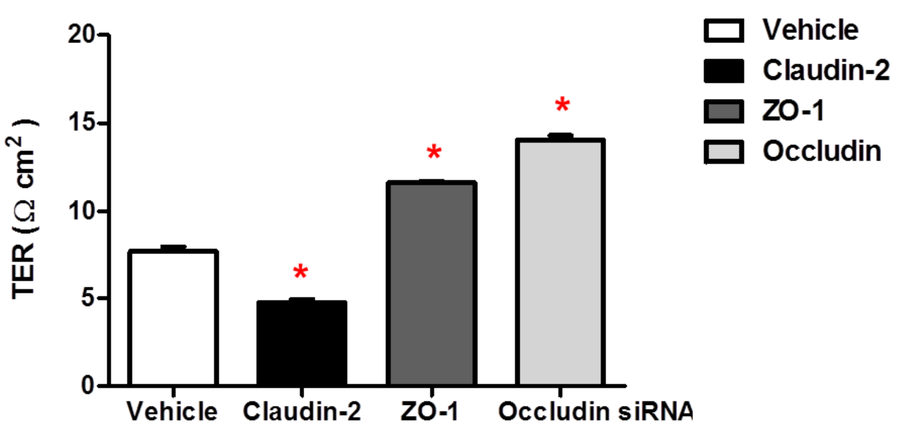
Small-interfering RNAs (siRNA) for claudin-2, ZO-1, and occludin were transfected into HK-2 cells using DharmaFect transfection reagents. At 25 nM of each siRNA, the efficiency of silencing was confirmed by immunoblot analysis. HK-2 epithelial monolayer resistance and paracellular permeability were estimated by measurements of the TER and determination of transepithelial flux rates of dextrans (4 and 70 kDa), respectively, after the siRNA transfection for 24 h.

Results

Results of siRNA-induced knock-down of TJ proteins

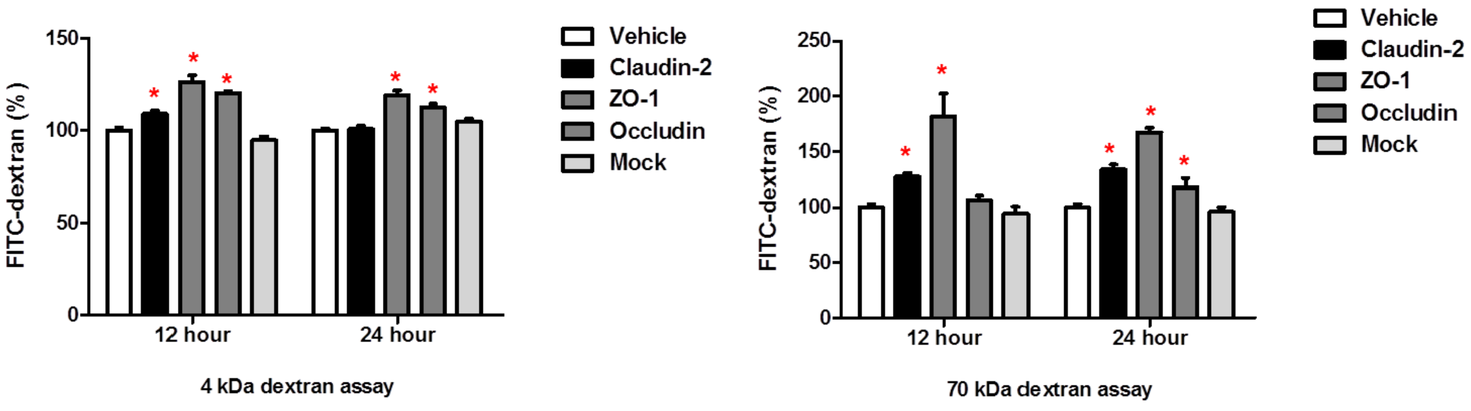


TER changes induced by a single TJ protein knock-down



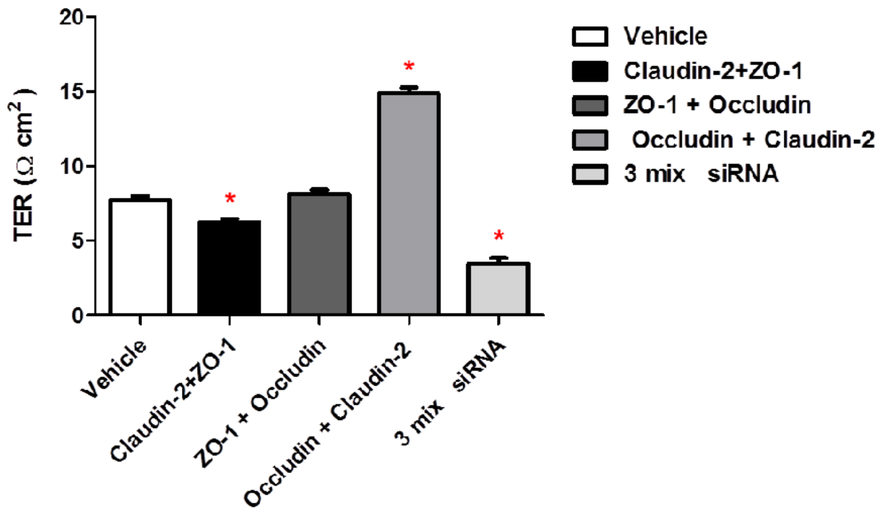
* $P < 0.05$ versus control by Mann-Whitney U test

FITC-dextran (4 & 70 kDa) permeability changes induced by a single TJ protein knock-down

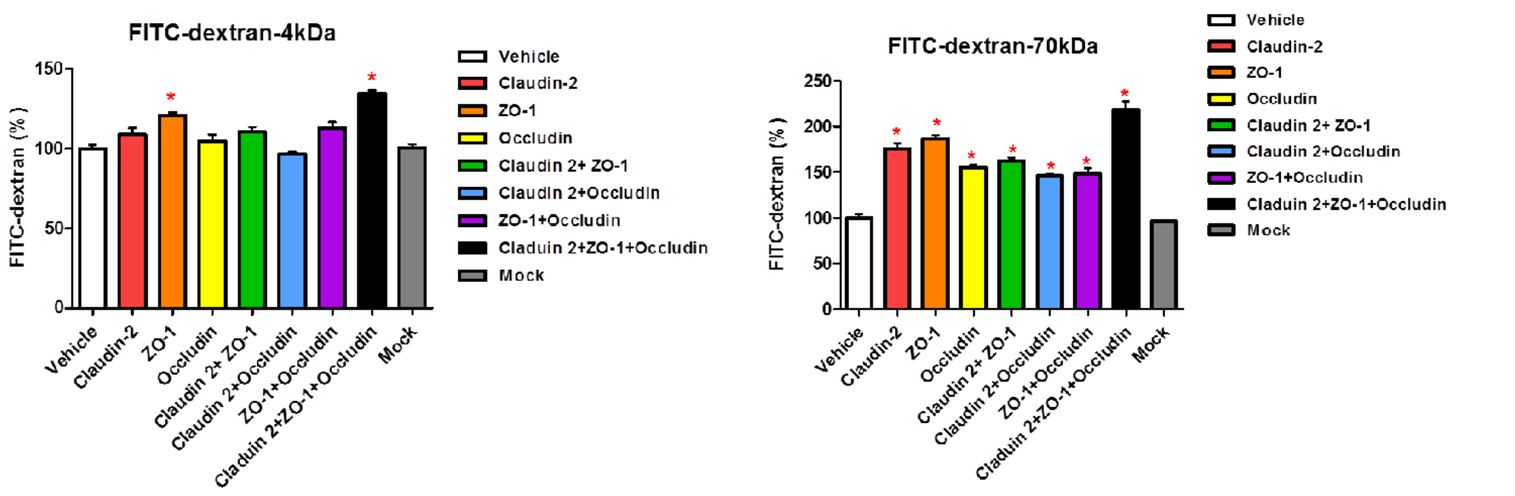


* $P < 0.05$ versus control by Mann-Whitney U test

TER changes induced by multiple TJ protein knock-downs



FITC-dextran (4 & 70 kDa) permeability changes induced by multiple TJ protein knock-downs



Immunoblots: Interaction between TJ proteins

Conclusion

These results show that depletion of claudin-2, ZO-1, and occludin in HK-2 cells leads to differential effects on TER and macromolecule flux. Interactions between claudin-2, ZO-1 and occludin seem to work for this leaky epithelium.

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A) Acid-base/Na, K, Cl, uric acid.

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