

## HK-2 세포의 밀착연결단백 claudin-2, ZO-1 및 occludin의 역할

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### The Roles of Claudin-2, ZO-1 and Occludin in the Leaky HK-2 Cells

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**Background:** 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 Dharma Fect 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:** The siRNA transfection of claudin-2, ZO-1, and occludin produced near-complete knock downs of respective TJ protein expression. The control HK-2 monolayers achieved a steady-state TER of  $6-8 \Omega \cdot \text{cm}^2$  when grown on 12-well Transwell filters, compatible with leaky epithelia. The siRNA-induced claudin-2 knock down produced a significant decrease in TER and an increase in occludin expression. On the other hand, either ZO-1 or occludin siRNA transfection resulted in a significant increase in TER and a decrease in claudin-2 expression. The dextran flux rate was significantly increased by claudin-2, ZO-1, or occludin siRNA transfection. The increase in dextran flux was enhanced by co-transfection of claudin-2, ZO-1, and occludin siRNA. TER showed different responses to siRNA co-transfection of claudin-2+ZO-1, ZO-1+occludin, and occludin+claudin-2, but it was remarkably decreased by co-transfection of claudin-2, ZO-1, and occludin siRNA.

**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.

**Key Words:** 밀착연결단백, 근위세관, HK-2 세포

Tight junction protein, Proximal tubule, HK-2 cell