Establishment and characterization of cells stably expressing DRA

Hisayoshi Hayashi and Yuichi Suzuki
Laboratory of Physiology, School of Nutritional Sciences, University of Shizuoka

Summary

It has been conceived that NaCl absorption in the gastrointestinal tract is mediated by coupled Na^+/H^+ and Cl^-/HCO_3^- exchangers. NHE3 is a major Na^+/H^+ exchanger contributing to NaCl absorption; however, the molecular identity of the Cl^-/HCO_3^- exchanger involved in NaCl absorption is uncertain. Recent studies have suggested that Down–regulated in adenoma (DRA, also called Slc26a3) is a major Cl^-/HCO_3^- exchanger in the colon. Since multiple isoforms of the Cl^-/HCO_3^- exchanger are co-expressed in an intact colonic cell, complicating the functional analysis of an individual isoform, we used heterologous transfection of DRA into cell lines. We generated a construct encoding the DRA tagged with the hemagglutinin (HA) epitope and transiently transfected the cells with this construct. The expression of the HA-tagged DRA by immunoblotting was verified. Two immunoreactive bands were detected in the transfected cells: a major protein of 120 kDa, probably glycosylated DRA, and a smaller band of 90 kDa, which is probably un-glycosylated DRA. This assumption was validated by treatment of the cells with an *N*-glycosylation inhibitor resulting in the disappearance of the higher molecular band. The subcellular localization of DRA was assessed by immunofluorescence experiments; DRA was found almost exclusively in the surface membrane. Since DRA expression was not stable and disappeared over weeks, we established the cells stably expressing DRA by using inducible gene expression systems. When the cells were induced DRA, the cells were conferred DIDS insensitive Cl^-/HCO_3^- exchange activity. Immunofluorescence experiments showed that colocalization of actin and DRA. DRA associates with regulatory factors called NHERF. In addition, this factor interacts with the cytoskeletal protein ezrin, which in turn binds to actin. The possible linkage of DRA with the cytoskeleton prompted us to test the effect of actin-modifying agents on DRA activity. However, latrunculin B, which interferes with actin polymerization, did not affect DRA activity.