Apical Recycling Endosome-Associated Myosin Vb is Mutated in Microvillus Inclusion Disease and Is Involved in Intestinal Brush Border Development

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Background & Aims: Microvillus inclusion disease is a rare and fatal congenital enteropathy, presenting with intractable secretory diarrhea shortly after birth. The complete inability to absorb nutrients from intestinal luminal demands total parental nutrition, and, eventually, transplantation of the small intestine. MID characteristics varies among patients and generally comprises of villous atrophy and crypt hyperplasia, and, at the cellular level, by the apical brush border atrophy, accumulation of apical proteins, lysosomes and microvilli-like inclusions in the apical cytoplasm of intestinal absorptive cells. Previously we have shown that MID enterocytes display abnormal expression of apical recycling endosomal markers, i.e Rab11a, FIP-1 (RCIP), FIP-5 (Rip11), resulting in a defective apical recycling system in MID. In this study, we aimed to identify the genetic cause and functional consequences that underlie the microvillus inclusion disease. Methods: We screened the genomic DNA of three patients diagnosed with microvillus inclusion disease, their siblings and parents. Biopsies of small intestine from MID and control patients were used to analyze the organization of organelles and localization of proteins involved in intraepithelial trafficking of both apical and basolateral proteins. Results: In all MID patients together we have identified two substitutions, one deletion, and two protein truncating mutations in the MYO5B gene. The MYO5B encodes for an actin filament-binding molecular motor protein that interacts with the small GTPase Rab11a, a marker of recycling endosome and functions to maintain the correct subcellular trafficking of both apical and basolateral membrane proteins. Results: In all MID patients together we have identified two substitutions, one deletion, and two protein truncating mutations in the MYO5B gene. The MYO5B encodes for an actin filament-binding molecular motor protein that interacts with the small GTPase Rab11a, a marker of recycling endosome and functions to maintain the correct subcellular trafficking of both apical and basolateral membrane proteins. Mutations occurring at different positions of MYO5B gene and thus affecting different functional regions of MYO5B protein could explain the diversity of phenotypes present in MID patients. The identification of mutations in MYO5B as the cause for MID brings a major advance in setting the reliable diagnosis, enables the genetic counseling and prenatal screening, as well as paves the way for developing alternative therapeutic strategies.

Intestine Specific Deletion of N-WASP Leads to Alteration of Gut Homeostasis in Mice

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Background: Wiskott-Aldrich Syndrome protein (N-WASP) is a cytoplasmic protein in hematopoietic and epithelial cells that regulates proteins involved in intracellular trafficking and cytoskeletal homeostasis. N-WASP, like its homolog WASP, is associated with BBD in humans and spontaneous colitis in mice, and recent genome-wide linkage studies provide evidence of novel susceptibility factors for UC in the region. Methods: To generate mice with gut-restricted deletion of N-WASP (intestinal N-WASP KO, iNWKO), mice expressing Cre recombinase under the control of the villin promoter (tg-v-cre) were mated to mice homozygous for a floxed N-WASP allele (N-WASPL2L/L2L). Intestinal epithelial cells were isolated by EDTA dissociation and centrifugation, and tissue was examined with H&E, Alcan blue, immunofluorescence and electron microscopy (EM). To examine proliferation and migration of N-WASP-deficient enterocytes, myosin Vb protein could explain the diversity of phentotypes present in MID patients. The identification of mutations in MYO5B as the cause for MID brings a major advance in setting the reliable diagnosis, enables the genetic counseling and prenatal screening, as well as paves the way for developing alternative therapeutic strategies.

Intestinal DCs and IL-10 Promote Actin Organization and Cell Migration in Vitro

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Background: Intestinal dendritic cells (DCs) are essential for the maintenance of gut homeostasis, and cell migration is critical for DC function. However, the role of actin cytoskeleton in intestinal DCs and its regulation by IL-10 remain unclear. We hypothesized that IL-10 regulates actin cytoskeleton and DC migration.

Methods: We cultured murine bone marrow-derived DCs in the absence or presence of IL-10 for 4 days. We quantified actin cytoskeleton by phalloidin staining and DC migration by a transwell assay.

Results: IL-10 significantly increased phalloidin staining and promoted DC migration, which was diminished in DCs from IL-10−/− mice. Pretreatment with Latrunculin A completely inhibited DC migration, whereas IL-10 did not affect cell growth in culture. In contrast, IL-10 inhibited cell migration of DCs in vitro, but not in vivo, where treatment with IL-10 did not affect DC migration in vivo. These results suggest that IL-10 regulates actin cytoskeleton and DC migration in vivo, but not in vitro, and highlight the importance of actin cytoskeleton in DC function.

Conclusions: These findings provide insights into the role of actin cytoskeleton in intestinal DCs and suggest potential therapeutic targets for the treatment of immune-mediated gastrointestinal disorders.

The ZO-1 Actin Binding Region (ABR) Is Required for Cytoskeletal Tight Junction Regulation

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Background: The tight junction is a critical component of the epithelial barrier and regulates paracellular permeability. The ZO-1 ABR is required for MLCK-dependent tight junction regulation. However, the mechanism by which the ABR regulates tight junction function remains unclear.

Methods: We demonstrate that the ABR is required for MLCK-dependent tight junction regulation. The ABR regulates tight junction function by interacting with ZO-1 and N-WASP, and by regulating actin cytoskeleton dynamics. Moreover, the ABR is required for MLCK-dependent tight junction regulation in vivo.

Results: The ABR is required for MLCK-dependent tight junction regulation in vivo. The ABR is required for MLCK-dependent tight junction regulation in vivo. The ABR is required for MLCK-dependent tight junction regulation in vivo.

Conclusions: The ABR is required for MLCK-dependent tight junction regulation in vivo.