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Microvillus Inclusion Disease. Lessons about the apical plasma membrane.

Golachowska, Magdalena Renata

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Summary

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SUMMARY

A structurally, compositionally, and functionally distinct plasma membrane at the apex of the intestinal epithelial cell monolayer provides a selective and protective barrier that regulates the uptake of nutrients from the lumen. The inability of intestinal cells to develop such an apical brush border and the consequences it has on human functioning becomes particularly apparent in patients diagnosed with microvillus inclusion disease (MVID) (OMIM 251850), a rare hereditary enteropathy presenting with severe intractable diarrhea and malabsorption in neonates. At the cellular level, brush border atrophy with accumulation of lysosomal granules and microvillus inclusions is observed in the apical cytoplasm of MVID enterocytes. Apical brush border components are typically absent from the cell surface and accumulate in the apical cytoplasm. In contrast to the apical proteins, basolateral proteins display a normal polarized distribution at the surface of MVID enterocytes, which appear normally arranged in monolayers with distinguishable cell-cell adhesion junctions. Because of the specific loss of apical surface identity, MVID provides an outstanding opportunity to study the genetics and molecular dynamics that underlie apical surface development.

The aim of this thesis was to elucidate the genetic cause of microvillus inclusion disease (MVID) and obtain insight into MVID pathogenesis and, in this way, obtain insight into molecular mechanisms that control apical plasma membrane development and dynamics in general.

The genomic DNA of nine MVID patients was screened for mutations, and immunohistochemistry on three patients' material was performed to investigate resultant cellular consequences. We identified eight novel mutations in the *MYO5B* gene in all nine patients (**Chapter 2**). The *MYO5B* gene is ubiquitously expressed in all epithelial cells and encodes for a recycling endosome-associated and actin filament-binding motor protein. Importantly, we observed aberrant expression and subcellular distribution of the mutant myosin Vb protein in MVID enterocytes. Moreover, we demonstrate that the typical and myosin Vb-controlled accumulation of

Rab11a- and FIP5-positive recycling endosomes in the apical cytoplasm of the cells is abolished in MVID enterocytes, which is indicative for altered myosin Vb function. The data indicate that the apical endosomal system that ensures the recycling of apical brush border proteins, with myosin Vb as a critical regulator, is required to develop the apical cell surface in human enterocytes, and perturbations in this may be part of the pathogenesis of microvillus inclusion disease.

We reported two MVID patients that also developed renal Fanconi Syndrome (**Chapter 3**). Renal Fanconi Syndrome has been correlated to apical plasma membrane defects in the proximal tubular epithelial cells of the kidney. We therefore determined whether *MYO5B* mutations in these patients correlate with similar apical plasma membrane defects in renal tubular epithelial cells as observed in the intestine. Immunohistochemistry and/ or transmission electron microscopy analyses of biopsies from kidney, duodenum, ileum, jejunum and colon revealed that structural defects of the brush border and apical recycling endosome organization is observed in enterocytes of all segments of the small intestine and colon. *MYO5B* mutations in MVID patients with renal Fanconi syndrome do not correlate with aberrant apical plasma membrane morphology or altered apical recycling endosome organization in renal tubular epithelial cells. It is concluded that *MYO5B* mutations have divergent effects on the apical membrane system in kidney and intestinal epithelial cells. The epithelial defects presented in MVID are therefore likely triggered by intestine-specific (possibly environmental) factors, the identification of which may provide new targets and open new avenues for the development of alternative therapeutic strategies to combat this devastating disease.

One environmental factor that may be common to MVID and other pathological conditions associated with apical plasma membrane inclusions is extracellular acidosis. Therefore, we investigated whether extracellular acidosis may contribute to the formation of apical plasma membrane inclusions (**Chapter 4**). Using live cell imaging, we demonstrated that extracellular and cytoplasmic acidosis induced thinning/stretching and retrieval of large parts of the apical plasma membrane, while

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basolateral plasma membranes and cell-cell junctions remained unaffected. Internalized apical membranes were shown to be able to fuse with each other, giving rise to larger cytoplasmic vacuolar compartments. Compounds that interfered with actin filament dynamics similarly induced apical plasma membrane retrieval, and acidosis-induced apical membrane retrieval required Rho kinase and myosin-II. These data indicated that acidosis can induce a Rho kinase/myosin-II-dependent selective retrieval of the apical plasma membrane and formation of intracytoplasmic apical vacuoles.

We then investigated the effect of extracellular acidosis on the structure and function of the Golgi apparatus, an organelle that plays an important role in the development and maintenance of function apical plasma membrane domains (**Chapter 5**). We show that extracellular acidosis or direct intracellular acidosis caused a reversible temperature-dependent swelling and fragmentation of the Golgi apparatus, the extent of which correlated with decreasing pH values and exposure times. Acidosis did not prevent the *de novo* biogenesis of Golgi membranes, albeit fragmented, following brefeldin A withdrawal. Surprisingly, no intracellular accumulation of apical plasma membrane proteins was observed. Nevertheless, newly synthesized apical plasma membrane proteins passed through the fragmented Golgi membranes, as evidenced by their low temperature-mediated accumulation in fragmented Golgi membranes. Furthermore, the metabolism of a ceramide analog and the polarized transport of its newly synthesized metabolites to the apical surface were not inhibited. These data suggested that the overall structure and apical positioning of the Golgi complex can be uncoupled from its function in apical plasma membrane delivery. It is therefore unlikely that loss of Golgi structure is responsible for the observed acidosis-induced retrieval of the apical plasma membrane.

In conclusion, the genetic cause of microvillus inclusion disease (MVID) was elucidated and insight into the pathogenesis of MVID and, more generally, into molecular mechanisms that control apical plasma membrane development and dynamics in general was provided.

DISCUSSION AND PERSPECTIVES

In this thesis we demonstrate structural and functional changes in the apical plasma membrane which reflect internal and external pathological conditions.

Microvillus inclusion disease (MVID) may be correlated with an affected function of the apical recycling system due to myosin Vb protein structural aberrations, as all studied patients (except for one) carry mutations in the *MYO5B* gene. Plausibly, a defective apical recycling system accounts for the loss of resident proteins typically exposed at the brush border membrane. In addition, apical recycling endosomes may carry enzymes at their cytoplasmic surface that control brush border microvilli development, and this may be inhibited in MVID enterocytes.

Despite being a single-gene disease, MVID presents with a wide spectrum of symptoms varying among different patients. The severe secretory diarrhea, which is the first clinical presentation, starts within a few days (early onset) or few weeks (late onset) after birth. The microvillus inclusions, which gave the name to the disease, in most of the patients, are found in duodenal enterocytes. However, other parts of the gastrointestinal tract might be affected as well. The percentage of enterocytes that actually contain these inclusions also greatly varies among patients, i.e., from 10% to, in some cases, none. Similarly, MVID patients differ in the level of brush-border atrophy, as well as villus atrophy.

Until now the limited number of screened patients does not allow us to make a careful genotype-phenotype correlation. However, it is not unlikely that the diversity in MVID presentation can be correlated with the type of mutation affecting the myosin Vb protein in a different way. The mutations on both alleles (e.g. homozygous) that result in complete absence of the myosin Vb protein (e.g. via nonsense-mediated mRNA decay) might be responsible for severe symptoms (e.g. Patient 8, Chapter 2), while the heterozygous substitution mutations that change a single aminoacid in the myosin Vb motor domain may result in altered (but still present) myosin/actin interactions and reflect less severe disease outcome/progression (e.g. Patient 9, Chapter 2).

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Myosin Vb protein is ubiquitously expressed in epithelial cells. However, the structural and functional apical membrane defects are, thus far, found only in the intestinal epithelium. There can be several explanations for this. First, malfunctioning of other organs might be concealed by the general absorption defects and/or side-effects of the treatment (e.g. total parenteral nutrition that ceases the bile salt secretion, while being detrimental to kidneys and liver). Furthermore, there are suspicions of treatment-independent MVID liver malfunction following intestinal transplantation when the intrahepatic circulation is restored (our unpublished observations). Secondly, there might be intestine-specific intrinsic factors involved, e.g. relative expression levels of myosin Vb versus myosin Va and/or Vc, or yet unknown intestinal myosin Vb-interacting partners. Moreover, a very high rate of apical membrane dynamics (endo-, exocytosis and recycling) specific for short-living intestinal absorptive cells might intensify and accelerate the time of appearance of the structural and functional defects. In addition to intrinsic factors, organ-specific environmental factors might be involved. The duodenum contributes to stomach-derived acid neutralization by e.g. mixing acid with bicarbonate, secreted by $\text{Cl}^-/\text{HCO}_3^-$ exchanger (coupled to NHE-3 and CFTR). Despite ceased oral nutrition in MVID patients, their stomach might continue to excrete acid upon neuronal and hormonal stimulation. Lack of the apical membrane proteins, including proton exchangers (due to brush-border atrophy and apical recycling defects) in the duodenum of MVID patients may hamper the acid neutralization process and result in enterocyte acidification. The prolonged exposure of the apical side of the intestinal epithelial cells to low pH might serve as additional factor for the structural and functional changes in the enterocytes. Different treatments of MVID patients (with some receiving acid-neutralizing medication) possibly reflect disease severity and the level of structural abnormalities. Studying the correlation between particular myosin Vb mutations, as well as detailed comparison of treatments of MVID-diagnosed patients may shed new light onto the disease mechanism and symptoms presentation and result in modification of the routine treatment towards the tailor-made treatment.