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EDITORIAL

Modeling Microvillus Inclusion Formation In Vitro

The human genetic disorder, microvillus inclusion disease (MVID), is characterized by life-threatening diarrhea.[1] Although MVID is a rare condition, the severely limited treatment options available to patients and the high mortality rate attest to the need for elucidating molecular mechanisms that underpin MVID. Mutations in Myosin Vb (MYO5B) give rise to MVID.[2,3] MYO5B regulates proper trafficking of transporters and enzymes to the apical membranes of intestinal enterocytes. Similarly, Syntaxin 3 (STX3) is a SNARE protein that regulates apical trafficking in a number of epithelial cell systems. While inactivating mutations in MYO5B lead to the formation of intracellular inclusions, the syntaxin binding protein 2 (STXBP2), also known as MUNC18-2, forms a complex with STX3 to regulate apical membrane transport and fusion in intestinal epithelium.[4] Loss-of-function mutations in STXBP2/MUNC18-2 cause familial hemophagocytic lymphohistiocytosis type 5 (FHL5) disorder. FHL5 is characterized as an immune system disorder; however, patients with FHL5 present with a spectrum of gastrointestinal disorders, many of which resemble cellular abnormalities seen in MVID.[5] The broad spectrum of gastrointestinal disorders in patients with MVID and FHL5 demonstrates the need for improved and minimally invasive methods of delineating intestinal epithelial abnormalities that arise from genetic mutations. Although analysis of patient biopsies is valuable, the limited availability of tissue for evaluation and manipulation poses difficulties. With the advent of epithelial cell cultures known as intestinal organoids/enteroids since 2009,[6] researchers now have a novel tool to examine multiple aspects of cell biology without relying on cancer cell lines.

The research presented by Mosa et al[7] in the current issue of *Cellular and Molecular Gastroenterology and Hepatology* uses intestinal organoids derived from MUNC18-2 knockout mice to evaluate the formation of microvillus inclusions in differentiated enterocytes that have disrupted apical trafficking. Intestinal organoid cultures hold numerous advantages over transformed cell lines including self-renewal capacity, full compendium of differentiated cell type, crypt-villus architecture, and physiological capabilities. In addition, unlike cancer cell lines such as Caco-2, enterocytes in intestinal organoids display the full compendium of apical transporters and enzymes found in a normal enterocyte in intestinal tissue. Loss of Stxbp2/Munc18-2 results in embryonic lethality in mice. To circumvent this limitation, the authors of this article generated small intestinal organoids from mice with a homozygous conditional Munc18-2 null allele. Administration of lentiviral Cre to these organoids established stable Munc18-2 knockout intestinal organoids. Mosa et al[7] report that Munc18-2 knockout intestinal organoids have abnormal subapical localization of alkaline phosphatase and CD10, shortened microvilli, and an accumulation of secretory vesicles. The defects in the Munc18-2 knockout intestinal organoids were fully rescued by re-expression of wild-type human MUNC18-2 protein. Stable expression of mutant human patient MUNC18-2 variants was unable to restore enterocyte defects in Munc18-2 knockout intestinal organoids. These findings closely mirror the intestinal phenotype of FHL5 patients, suggesting that experimental use of intestinal organoids may be crucial for designing therapeutic strategies for treating congenital disorders.

Previous to this study, the origin of microvillus inclusions in MVID, whether from endocytosis or exocytosis, has been controversial. Previous studies in in vitro systems and in MYO5B knockout mice had suggested that inclusions were forming from endocytosis.[8,9] Mosa et al[7] performed live imaging of differentiated Munc18-2 deficient intestinal organoids to analyze microvillus inclusion formation. They convincingly report that F-actin positive foci develop into microvillus inclusions, which increase in size and number as enterocytes mature/differentiate. The authors also demonstrate that microvillus inclusion formation is dynamic, resulting from apical or basolateral membrane internalization or de novo in the cytoplasm. The results in this investigation suggest that microvillus inclusion formation with Munc18-2 loss is heterogeneous, reflecting multiple pathways. Nevertheless, it is important to note that the inclusions forming internally appear to be generally smaller than those that develop from endocytosis from the apical and basolateral membranes. It will therefore be important to evaluate in greater depth whether particular endocytotic events predominate in situ in tissues. Such studies will likely require the development of inducible intestine-specific knockout mouse strains.

This study by Mosa et al[7] highlights the utility of intestinal organoids in studying complex diseases that have no viable mouse model. It also demonstrates the likely value of using biopsies from patients to generate human intestinal organoids to tailor treatment strategies and elucidate epithelial alterations, especially in disorders that exhibit phenotypic spectrums such as FHL5 and MVID. The work by Mosa et al[7] raises some unique questions on the mechanism of microvillus inclusion formation and whether this pathway is dysregulated in disease and exaggerated by loss of Munc18-2. Because STX3-dependent pathways are critical for general apical transport in epithelial cells, it remains to be determined whether microvillus inclusions formed in enterocytes with loss of Munc18-2 or MYO5B are

direct causes of the diarrhea in patients or in mouse models.

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Conflicts of interest
The authors disclose no conflicts.


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