

Background: The elaborate apical membrane of intestinal enterocytes consist of actin rich protrusions called microvilli. Microvilli dramatically increase the surface area of the intestine maximizing absorption. Each enterocyte has about 1000 microvilli lining their apical membrane, collectively known as the brush border. To accommodate so many microvilli on a single cell, microvilli are densely packed and highly organized. This organization is orchestrated by the intermicrovillar adhesion complex (IMAC). IMAC is composed of protocadherins, protocadherin 24 (CDHR2) and mucin like protocadherin (CDHR5), as well as scaffolding proteins USH1C, ANKS4B and Myosin 7b (Myo7b). The IMAC forms physical adhesion links at the tips of microvilli to control packing and microvillar length. Defects in IMAC results in stunted microvilli and has been linked to Crohn's disease. To create an IMAC complex, cells traffic proteins to the apical membrane. Myosin 5b (Myo5b) is a molecular motor that is critical for delivering ion transporters to the apical membrane of enterocytes. Loss of functional Myo5b results in decreased apical expression of transporters and profound diarrhea in animal models and humans. We hypothesized that Myo5b is responsible for IMAC proteins localization.

Methods & Results: We used 2 different mouse models: neonatal germline Myo5b knockout (Myo5b KO) mice and adult intestinal specific tamoxifen inducible VillinCre^{ERT2};Myo5b^{flox/flox} mice. RNA sequencing showed decreased expression of CDHR2, CDHR5, USH1C and ANKS4B in germline Myo5b KO mice compared to littermate control mice. In control mice, immunostaining revealed that CDHR2, CDHR5, USH1C, and Myo7b were highly enriched at the microvilli tips. In contrast, neonatal germline and adult Myo5b deficient mice showed loss of apical CDHR2, CDHR5, and Myo7b in the brush border and accumulation in a subapical compartment compared to littermate controls. Co-localization analysis of CDHR2, CDHR5 and the lysosomal marker Lamp1 showed an increase in CDHR2 and CDHR5 in Lamp1 positive lysosomes; suggesting that CDHR2 and CDHR5 may be improperly trafficked for degradation in Myo5b KO mice. In neonatal Myo5b KO mice USH1C was found on the apical membrane of germline Myo5b KO mice, but did not appear enriched at microvilli tips. However, adult inducible Myo5b deficient mice showed a more complete loss of apical USH1C. Co-localization analysis for the microvilli marker gamma actin and each IMAC component revealed decreased Mander's coefficients in adult inducible Myo5b deficient mice compared to control mice for CDHR2, CDHR5, USH1C and Myo7b. Fourier's analysis further demonstrated aberrant microvilli packing in adult inducible Myo5b deficient mouse small intestine. **Conclusions:** These data indicate that Myo5b is responsible for the delivery of IMAC components to the apical membrane and thus controls the proper brush border formation and packing of microvilli.