Plectin Confers Mechanical Stability of Simple Epithelium

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Plectin is large, ubiquitously expressed protein from plakin family that interconnects all three cytoskeletal systems and links them to plasma membrane-bound junctional complexes. Plectin acts as a crosslinker and signalling scaffold that serves essential functions in maintenance of cell and tissue cytoarchitecture. To understand the specific role of Plectin in maintenance of epithelial mechanical stability, we generated liver-specific Plectin KO mice and Plectin-depleted epithelial cell lines.

In mice, Plectin deletion in hepatocytes and biliary epithelial cells caused aberrant organization of keratin filaments, biliary tree malformations and collapse of bile ducts and ductules. Plectin-deficient biliary epithelial cells formed compromised tight junctions, exhibited increased expression of the adherens junction protein E-cadherin and were inefficient in desmosomal protein desmoplakin upregulation upon experimentally induced cholestasis. Moreover, Plectin KO mice showed higher susceptibility to cholestatic challenge, induced by bile duct ligation, where Plectin-deficient bile ducts were prominently dilated with more frequent ruptures.

To complement the data obtained from mouse *in vivo* models, we generated Plectin-deficient epithelial cell lines (cholangiocytes and MDCKs), using the CRISPR-Cas9 technology. Plectin-deficient cholangiocytes showed impaired functional integrity of intercellular junctions, measured by transepithelial electrical resistance and monolayer permeability for FITC-dextran upon hyper-osmotic shock. Using supperesolution microscopy, we have shown that Plectin depletion causes collapse of keratin filaments, associated with irregularly shaped and partially bended desmosomes. Moreover, mechanical stretching of monolayers of MDCK cells also revieled higher susceptibility of Plectin KO cells to stretch-induced widening of desmosomal plaques, suggesting lower mechanical integrity of KO epithelial sheets.

These data demonstrate that Plectin is required for proper keratin network cytoarchitecture, correct formation of cell-cell junctions and epithelial stability both *in vitro* and *in vivo*.