5735
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Role of differential phosphorylation of JAM-A in regulating epithelial barrier function

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Junctional adhesion molecule-A (JAM-A) is an epithelial tight junction protein that has been shown to play an important role in regulating intestinal permeability through association with a scaffold signaling complex containing ZO-2, Afadin and the small GTPase Rap2. A previous study identified serine phosphorylation of the cytoplasmic tail of JAM-A (p-S284) and implicated its role in stabilizing tight junctions in MDCK cells. Here, we report that the cytoplasmic tail of JAM-A is tyrosine phosphorylated (p-Y280) under inflammatory conditions associated with loss of barrier. While minimal Y280 phosphorylation was observed in model intestinal epithelial monolayers with assembled intercellular junctions, serine phosphorylation was robust. Conversely, exposure of epithelial cells to cytokines IL-22 or IL-17a resulted in time dependent increase in p-Y280 that paralleled compromised epithelial barrier function assessed by measurement of transepithelial resistance to passive ion flow or paracellular flux of 4kDa dextran. Consistent with these in-vitro findings, in normal colonic mucosa, there was prominent serine phosphorylation but no tyrosine phosphorylation of JAM-A. However, the opposite was observed in colonic mucosa of individuals with ulcerative colitis and in mice with experimentally induced colitis (DSS) where serine phosphorylation was lost and tyrosine phosphorylation was robust. Coimmunoprecipitation and knockdown experiments identified a role of Src kinase Yes-1 in mediating tyrosine phosphorylation of JAM-A. Consistent with these findings, treatment of epithelial monolayers with the Src kinase inhibitor PP2 was able to partially rescue the barrier defect associated with cytokine exposure and tyrosine phosphorylation of JAM-A (p-Y280). Analysis of protein interactions with JAM-A under conditions of tyrosine phosphorylation revealed loss of tight junction-associated p-JAM-A Y280 and scaffold proteins ZO-2 and active Rap2 in parallel with compromise in the epithelial barrier function. These results suggest that JAM-A mediated regulation of intestinal epithelial permeability is dependent on a delicate balance between serine and tyrosine phosphorylation events that are altered during inflammation resulting in a leaky barrier.