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Inhibition of Protein Translation Initiation and Disruption of the Intestinal Epithelial Barrier in Mucosal Inflammation

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Integrity of the intestinal epithelial barrier is regulated by two types of adhesive structures: intercellular junctions and focal adhesions (FA) that control epithelial cell attachment to each other and to the extracellular matrix (ECM), respectively. Disassembly of adherens junctions (AJ) and tight junctions (TJ) plays key roles in the breakdown of the intestinal epithelial barrier during mucosal inflammation. Abnormal architecture and functions of FA significantly contribute to attenuated restitution of the inflamed intestinal mucosa. Proinflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, are known to trigger AJ/TJ disassembly and attenuate wound healing in model intestinal epithelial cell monolayers. We hypothesized that inhibition of protein translation factors eIF4G1 and eIF4G2 could mediate cytokine-induced disruption of the gut barrier and attenuation of epithelial restitution.

Immunoblotting analysis of IFNγ and IFNγ/TNFα-treated HT29 and T84 human colonic epithelial cells demonstrated significant downregulation of expression AJ proteins, E-cadherin, p120 catenin, β-catenin, and the TJ protein, occludin. By contrast quantitative RT-PCR analysis did not reveal changes in mRNA levels of selected junctional proteins. Furthermore, expression of translation initiation factors eIF4G1 and eIF4G2 was downregulated by these proinflammatory cytokines *in vitro,* and eIF4G2 level was decreased in colonic mucosa of IBD patients. In order to examine functional roles of these translation initiation factors, eIF4G1 or eIF4G2 were selectively downregulated in well-differentiated SK-CO15 and HT29cF8 human colonic epithelial cell lines, as well as in stem-cell like human intestinal epithelial cells (HIEC) by using either siRNA-mediated or shRNA-mediated knock-down. Downregulation of either eIF4G1, or eIF4G2 increased permeability of SK-CO15 epithelial cell monolayers and decreased expression of different adherens junction and tight junction proteins. Furthermore, depletion of these translation initiating factors inhibited collective wound healing and individual transfilter migration of both stem-cell like and well-differentiated intestinal epithelial cells. Interestingly, immunolabeling of newly-translated proteins identified the epithelial leading edge as the hot-spot site of active translation during wound healing. Finally, we observed a robust epithelial to myofibroblast transition in HIEC cells upon depletion of eIF4G1. The described results reveal a novel role for protein translation initiation in regulating intestinal epithelial barrier and epithelial restitution and suggest that this important homeostatic mechanism could be impaired during mucosal inflammation and IBD.

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