Characterizing the Role of the E3 Ligase ITCH in Gut Mucosal Homeostasis

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The mucosal barrier of the small intestine is highly dynamic, enabling the passage of essential nutrients that are necessary for the body's function while simultaneously preventing a breach of harmful microorganisms that are damaging to the host. The effectiveness of the mucosal barrier is dependent on the cohesive relationship established between the outer mucosal epithelium and the underlying immune compartment in the small intestine. The epithelium provides the first line of defense against pathogens by establishing a physical barrier separating the external environment from the body's internal milieu, while the immune system secondarily responds to clear bacteria that have breeched the epithelial barrier. The HECT E3 ubiquitin ligase ITCH is known to regulate immune responses, and loss of function of ITCH has been associated with gastrointestinal inflammatory disorders. However, the high level of ITCH expression within the intestinal epithelium suggests that it may have important function in that tissue as well to maintain gut homeostasis. Indeed, we identified that global loss if ITCH (*Itch*$^{a18H/a18H}$) in young adult animals resulted in altered intestinal homeostasis characterized by increases in both crypt and villus area that were more prominent in the distal part of the small intestine. Increased crypt area was found to result from expansion of both the proliferating transit amplifying (TA) progenitor population and terminally differentiated Paneth cells. Lack of ITCH also resulted in changes in numbers of terminally differentiated cells on the villus, with increases in goblet cells, decreases in enterocytes, but no change in enteroendocrine cells. Epithelial cell turnover was also accelerated in *Itch*$^{a18h/a18H}$ animals supported by increases in both proliferation and apoptosis within the crypt, as well a more rapid cell migration of bromodeoxyuridine-labeled epithelial cells along the crypt-villus axis. Consistent with the observed enhancement of cellular migration, *Itch*$^{a18H/a18H}$ mice carrying the *Min* mutation (*Itch*$^{a18H/a18H}$; *Apc*$^{Min/+}$) displayed a 76% reduction in tumor burden as compared to *Apc*$^{Min/+}$ littermates with normal levels of ITCH. To identify the cell autonomous function of ITCH in epithelial homeostasis, intestinal organoids were generated from the crypts of ITCH deficient animals. Interestingly, epithelial cell proliferation and differentiation were not perturbed in ITCH deficient organoids, in contrast to the *in vivo* phenotype of the *Itch*$^{a18H/a18H}$ small intestines. However, increased apoptosis was observed in organoids lacking ITCH, which is consistent with increased cleaved-caspase 3 staining in the intestines of mice lacking ITCH exclusively in the intestinal epithelium. The failure to recapitulate the *Itch*$^{a18H/a18H}$ epithelial phenotype prompted us to investigate the impact loss of ITCH function in immune cells has on the epithelium. Animals lacking ITCH within the myeloid cell lineage have similar defects in crypt area, as well as increases goblet and Paneth cell numbers, as compared to the *Itch*$^{a18H/a18H}$ animals, albeit delayed. These finding highlight a cell autonomous as well as non-cell autonomous function for ITCH in mediating epithelial homeostasis, and emphasize the importance of ITCH in small intestinal barrier function.