

Abstract

Gut barrier integrity requires constant transcriptional adaptation of intestinal epithelial cells (IECs) to signals from immune cells, microbiota, IEC-crosstalk, and diet. In obesity, pro-inflammatory cues might alter these homeostatic transcriptional programs in IECs. Hitherto, the spatial landscape of obesity-induced response mechanisms of the intestinal epithelium is not well explored. In this study, cell type-specific transcriptional adaptations in IECs from duodenum, jejunum, ileum, and colon of high-fat diet (HFD)-fed C57BL/6N mice were investigated using single-cell transcriptomics. HFD-induced dysregulation of gene expression displayed regional specialization between the intestinal segments. We identified a novel cluster of HFD-associated enterocytes (HAEs) in the colon, which we aimed to examine via intersectional genetic strategies based on *Cre/loxP* and *Dre/rox* systems. Although novel *Vil1-2A-DD-Dre* and *Plb1^{E2A-CreERT2}* mice failed to label HAEs, upon adaption, we were able to partially target specific subpopulations of enteroendocrine cells. Interestingly, *Plb1* expression marked differentiated enterocytes in the ileum. *Plb1*⁺ IECs displayed HFD-feeding-induced expression of major histocompatibility complex class II (MHCII) antigen presentation pathway genes due to a dynamic shift of intestinal microbiota. Functional verification experiments in IEC-specific CD74 or MHCII deficient mice revealed disrupted mucosal immune cell homeostasis. We observed an altered intestinal cytokine expression profile affecting the development of diet-induced insulin resistance. Collectively, the unique single-cell RNA sequencing dataset generated in this study serves as a powerful resource to distinguish region- and diet-specific adaptations of IECs, providing valuable insights into obesity-induced alterations of the intestinal transcriptome.