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Mitochondrial dysfuntion in intestinal epithelial cells influences regenerative and neoplastic processes

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Purpose: Tissue homeostasis in the intestinal epithelium requires a fine-tuned regulation of cell proliferation and differentiation. Phenotypic transitions of intestinal epithelial cells (IECs) occur along the crypt-villus axis, which are reflected by altered mitochondrial activity. Disturbances in mitochondrial function, metabolism and mitochondrial unfolded protein response (MT-UPR) have been implicated in various pathologies including inflammatory bowel disease (IBD) and cancer. We have recently shown that deletion of the mitochondrial chaperone Hsp60 in IECs activates MT-UPR and controls proliferation. Here, additional mouse models were used to further characterize mitochondrial dysfunction in disease development.

Methods: Several mouse models, including $HSP60^{flox/flox}$ x $VillinCreER^{T2}$, $ClpP^{flox/flox}$ x $VillinCreER^{T2}$, $IL10^{-/-}$ and $ATF6^{tg/tg}$ x VillinCre mice (all C57BL/6), were used. Colonic tissue sections were analyzed via immunofluorescence/chemistry staining of Hsp60, Wnt10a, Ki67 and BrdU. F4/80 and CD3 staining was performed to analyze immune cell infiltration. Furthermore, alterations in gene expression levels of epithelial subtypes, including stem cells, TA cells, Paneth cells and goblet cells, were assessed by RT-qPCR analysis.

Results: Underlying the relevance of MT-UPR and associated Hsp60 in healing processes, an induction of Hsp60 in hyperproliferative wound areas following mechanical and chemical wounding (DSS) was shown. Immunofluorescence staining of Hsp60 further illustrated its induction in inflammation-associated ($IL10^{-/-}$ mice) and genetically-driven tumor models. Reflecting the importance of these signaling processes, Cre-mediated loss of Hsp60 in colonic IEC ($Hsp60^{\Delta/\Delta IEC}$ mice) was followed by occurance of Hsp60+, Ki67+, BrdU+ hyperproliferative IEC foci, repopulating the colonic epithelium from day 5 after end of tamoxifen treatment. Concomitantly, an increased recruitment of immune cells, including macrophages, was observed. Moreover, IEC-specific deletion of the MT-UPR associated mitochondrial protease *ClpP* ($ClpP^{\Delta/\Delta IEC}$ mice) and mitochondrial metabolism-associated *Ckmt1* ($Ckmt1^{-/-}$ mice) depicted alterations in the intestinal stem cell niche, production of antimicrobial peptides and metabolic processes.

Conclusion: These data demonstrate a strong link between mitochondrial function, healing processes and neoplastic changes in intestinal epithelial cells. To better illustrate MT-UPR as a promising target in the context of disease, we will further analyze mitochondrial dysfunction in mouse models of sporadic and inflammation-associated cancer. Additionally, mechanistic processes will be elucidated using transcriptional profiling, metabolomics and analysis of stemness in murine intestinal organoids.