Epithelial and subepithelial players in chronic intestinal disease

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Summary

The intestinal epithelium and the subepithelial layers form the primary barrier in the human gut, that serves 2 main functions: 1) absorption of essential nutrients from the diet, but at the same time 2) protect against potentially harmful and/or infections substances in the gut lumen. Many chronic diseases are associated the toxic effects of these harmful substances on the intestinal epithelium and subepithelial layers and lead to intestinal dysfunction, such as intestinal cancer, chronic intestinal inflammation and fibrosis. Cigarette smoking is a well-known risk factor for developing colon cancer and Crohn's disease (CD), but it is less-well known that concentrations cigarette smoke-derived compounds are often higher in the gut lumen than in the blood stream of people who smoke. Thus, this is a serious threat for the gut epithelium and subepithelial layers. However, in what way cigarette smoke directly affects the human intestinal epithelial and subepithelial cells functioning is not really known. Smoking also leads to a more severe disease course of CD, which is characterized by a higher risk for developing fibrotic disease in the gut. This thesis focuses on the effect of cigarette smoke on the human intestinal epithelium and subepithelial cells (myofibroblasts) and analyzed the effect of an antifibrotic drug, pirfenidone, on the fibrogenic potential of intestinal myofibroblasts.

Chapter 2 employed the human intestinal organoid and 3-dimensional (3D) Caco-2 cell models to investigate the effects of CSE on epithelial cell morphogenesis, regeneration, polarity and barrier function. We show that exposure of intestinal epithelial cells to cigarette smoke extract (CSE) resulted in defective proliferative and differentiation function of intestinal stem cells and impaired polarization of intestinal enterocytes, leading to aberrant intestinal cell morphogenesis and functioning. The effects induced by CSE was transferred to next generation cells, e.g. appeared to induce irreversible effects. However, CSE did not perturb the epithelial polarity development or impair the epithelial barrier function of organoids, suggesting that intestinal epithelial tissue is more resistant to CSE exposure than lung epithelial tissue. Moreover, CSE did not

perturb the morphogenesis and polarity of pre-formed Caco-2 spheroids, suggesting that the effects of CSE on intestinal enterocyte may be relevant to the morphogenesis and polarity reestablishment, such as during the regenerative wound healing response. This study demonstrates that short-term CSE exposure exerts long-term effects in intestinal epithelial cells that may underlie the increased risk for developing colon cancer.

Cigarette smoking is also a risk factor of chronic intestinal inflammation, such as in CD. In **Chapter 3**, we analyzed the effect of cigarette smoking/CSE on the expression of vascular adhesion protein-1 (VAP-1; encoded by *AOC3*) in IBD patients and primary human intestinal fibroblasts (p-hIFs) to determine whether the immunomodulatory VAP-1 is involved in the pathogenetic mechanism of intestinal inflammation and/or fibrosis. Colonic *AOC3* expression was significantly higher in CD patients who smoke, when compared to non-smoking CD patients. We found that colonic *AOC3* levels strongly correlated with *COL1A1* and *ACTA2* levels (markers of fibrosis) in intestinal tissue in CD patients. In line, VAP-1 co-localized with αSMA in the intestinal subepithelial myofibroblasts, which was dominantly expressed in fibrotic areas. In line with the patient data, CSE exposure induced *AOC3* expression in primary human intestinal fibroblasts (p-hIFs), however, it did not change *COL1A1* and *ACTA2* levels in these cells. Cigarette smoke is likely to aggravate intestinal inflammation and thereby promote colonic fibrogenesis in CD by inducing immunomodulatory VAP-1, but not by directly enhancing the fibrogenic phenotype of intestinal fibroblasts.

As an additional spin-off of **Chapter 3**, we newly-identified and characterized a subtype of enteroendocrine cells that expresses immunomodulatory VAP-1. Besides typical VAP-1 staining of the mucosal endothelial cells, smooth muscle cells and subepithelial myofibroblasts, in **Chapter 4,** we observed solitary VAP-1^{+} intestinal epithelial cells (IEC) in the healthy colon, ileum and jejunum and in uninflamed, inflamed and stenotic colonic tissue of IBD patients. By immunohistochemical analysis of serial tissue sections and immunofluorescence co-staining, we demonstrate that VAP-1 is expressed in a subtype of chromogranin A^{+}-enteroendocrine cells. *In vitro*, we

detected increased expression of *CHGA*/chromogranin A and *AOC3*/VAP-1 in differentiated human colonic organoids, both of which accumulated at the basolateral side of the organoid. Future studies need to investigate the functional role of VAP-1 in enteroendocrine cells, which might be related to hormone secretion and/or immune regulation.

Intestinal fibrosis is a severe complication of chronic intestinal inflammation and there is an urgent need for effective anti-fibrosis drug therapies. **Chapter 5** studied the effect of pirfenidone on p-hIFs. We show that pirfenidone dose-dependently inhibited p-hIF proliferation and the expression of extra cellular matrix (ECM) component markers, including the *COL1A1*, *COL3A1*, *COL4A1*, *COL6A1*, *FN1* and *ELN*. TGFβ1, a key cytokine in the development fibrogenesis, strongly induced collagen-I expression in p-hIF, which was effectively suppressed by pirfenidone. The inhibiting effect of pirfenidone on p-hIF proliferation and collagen I production was reversible. The mammalian target of rapamycin (mTOR) signaling pathway contributes to organ fibrosis, but this was not analyzed in detail yet for intestinal fibrosis. We demonstrate that pirfenidone acts through deactivating mTOR and the downstream p70S6K pathway in p-hIFs. We conclude that pirfenidone directly suppressed p-hIFs proliferation and collagen I production via the TGF-β1/mTOR/p70S6K signaling pathway, which provided a novel drug target for the treatment of intestinal fibrosis.

In conclusion, chronic intestinal disease, including inflammation, fibrosis and cancer strongly impacts the life of many patients with a heavy and increasing burden on health care systems. A better understanding of the underlying mechanisms is needed to develop effective therapeutic methods. Human intestinal organoids and primary human intestinal fibroblasts are available and promising models to investigate the physiological and pathological intestinal cell morphology and functioning in diseases derived from intestinal epithelium and sub-epithelium and for drug development.