Epithelial RAC1 niches in IBD: from barrier integrity to cytoskeletal plasticity

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The gastrointestinal tract forms the largest contact area with the environment and tightly regulated mechanisms are needed to maintain intestinal homoeostasis. The inner lining of the gastrointestinal tract—the monolayer of intestinal epithelial cells (IECs)—therefore is a key contributor to this delicate balance. Barrier disruption and epithelial leakage can provoke a virtuous circle, where an exaxerbated immune response against environmental factors and microbes can result in chronic inflammation.[1] Patients with IBD often present with epithelial leakage, moreover states of remission frequently present with sustained increased intestinal permeability.[3] The importance of epithelial disruption in susceptibility to IBD development is further supported by the observation that multiple IBD susceptibility genes encode proteins involved in limiting the penetration of bacterial symbionts into the mucosal site and involved in promoting bacterial killing.[4 5]

Already in the 1980s, transmission electron microscopy and freeze fracture electron microscopy revealed ultrastructural changes in the junctional complexes between IECs in biopsies from patients with IBD.[2]

The intestinal epithelium stands out for being the most proliferative tissue in the vertebrate body that renews itself every few days through a physiological circuit of cell renewal, differentiation, proliferation and physiological cell shedding. All of these processes are central to maintaining barrier integrity and mucosal homoeostasis.[6] On a subcellular level, the cytoskeleton is the central scaffold to epithelial integrity and stable cell–cell junctions are needed to control paracellular permeability.[7] During inflammation, proinflammatory mediators engender cytoskeletal rearrangements resulting in a breakdown of epithelial integrity and increased permeability.[8] Both small and large GTPases have been shown to be involved in cytoskeletal rearrangements in the context of chronic inflammation.[9 10] Previously, López-Posadas et al were able to make decisive progress in understanding cellular and molecular events underlying cytoskeletal plasticity. Using conditional murine models, small GTPases were shown to especially impact on small intestinal integrity whereas the large intestinal epithelium turned out to be less affected.[10]

In *Gut,* Martínez-Sánchez et al have addressed the impact of the prenylation enzyme geranylgeranyltransferase-1 (GGT-1) and RAC1 on cell shedding alterations and barrier integrity in IBD.[11] First, they took advantage of murine conditional GGTase and RAC1 knock-out models ($Pggt1b^{i\Delta IEC}$ and $Rac1^{i\Delta IEC}$) to study functional consequences of epithelial specific knockouts. Inhibition of prenylation within IECs ($Pggt1b^{i\Delta IEC}$ mice) resulted in increased intestinal permeability and barrier disruption over time accompanied by an inflammatory response in the small intestine. Intravital microscopy revealed impaired cell shedding dynamics finally resulting in cytoskeleton redistribution, cell overcrowding and altered actomyosin dynamics. Real time fluorescence deformability cytometry in GGTase-deficient IECs revealed alterations in cellular mechanics on a single cell level. Moreover, regulated protein expression on epithelial deletion of $Pggt1b$ suggested potential changes of intercellular junctions and a redistribution of Apical Junction Complexes. To determine whether the observed intestinal phenotype was caspase-mediated, Martínez-Sánchez et al applied elegant kinetics, ruling out apoptosis and also necroptosis and pyroptotic cell death as initial events but suggesting those as secondary mechanisms on cytoskeleton rearrangement. Also, altered proliferation as potential underlying mechanism was also only observed at late timepoints after induced $Pggt1b$ deletion.

To disentangle molecular mechanisms behind the observed phenotype, the authors concentrated on prenylation targets and identified RAC1 as a GGTase target critically involved in the observed phenotype. Strikingly, $Rac1^{i\Delta IEC}$ mice phenocopied intestinal pathologies found on conditional GGTase deletion, again predominantly in the small intestine. A reductionist in vitro approach using small intestinal organoids from both $Pggt1b^{i\Delta IEC}$ and $Rac1^{i\Delta IEC}$ mice enabled the identification of potential underlying mechanisms. Both GGTase and RAC1-deficient organoids displayed decreased cell viability as indicator of epithelial disruption and showed cytoskeleton rearrangement and altered cell mechanics therefore recalling the in vivo phenotype. Precious hints derived from RNASeq analysis in RAC1-deficient small intestinal organoids showing regulated expression of genes involved in response mechanisms to both bacterial and mechanical stimuli as well as immune responses.

This study than nicely turns the attention towards a potential impact on clinical practice. Tissue samples from patients with IBD displayed signs of impaired epithelial integrity and moreover indications of arrested cell shedding as seen in murine models. To close the line of arguments and unravel the linkage between cytoskeleton rearrangement and epithelial leakage, the authors moved to human two-dimensional organoids and cell lines. Treatment of Caco-2 and HT29 cells with the RAC1 inhibitor NSC-23766 or GGTI-298 resulted in decreased transepithelial resistance and correlated with actin redistribution. Therefore, Martínez-Sánchez et al were able to confirm the link between RAC1-dependent epithelial alterations and epithelial barrier function as observed in the inflamed gut, once more underlining its clinical relevance. RAC1 dysfunction in IBD tissue was further supported by elegant studies investigating the subcellular localisation along the crypt-villus axis. Given that RAC1 is a known activator of a protein complex known as the WAVE regulatory complex, the authors analysed WAVE proteins to investigate potential activations of RAC1-downstream pathways. WAVE proteins are indispensably involved in the dynamics of actin filaments within a cell and therefore cellular integrity[12 13] and its expression and stability was reduced on $Rac1$ deletion. The authors observed decreased WAVE1 expression in IBD tissue and most interestingly a changed expression pattern along the crypt-villus axis, suggesting distinct niches of RAC1 dysfunction in IBD. Proteasome inhibition partly rescued disturbed cell shedding events in RAC1-deficient small intestinal

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organoids, pointing towards the relevance of RAC1 function and downstream pathway activation, moreover underlining its inevitably involvement in cytoskeleton constitution and therefore IEC integrity in patients with IBD.

The novelty of the here presented work is not the role of Rac1 and cell integrity for intestinal homoeostasis, but rather that epithelial intrinsic alterations due to absent GGTase or RAC1 function lead to an intestinal phenotype reminiscent of alterations seen in IBD tissue. In addition, these GGTase-dependent and consecutively RAC1-dependent alterations were predominantly found in the small intestine, reemphasising that small intestinal inflammation is distinct from colonic inflammation and deserves to be studied separately in order to identify site-specific therapeutic targets.[14] Together here described RAC1 function, therefore, represents a promising, attractive target for future diagnosis and treatment algorithms in particular for small intestinal Crohn's disease.

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