Chymase/Mcpt4: A serine protease with a crucial role in the modulation of colonic epithelial cell permeability in colitis

Afshin Derakhshani
Calvin, Phoebe and Joan Snyder Institute for Chronic Diseases; McCaig Institute for Bone and Joint Health, Cumming School of Medicine, University of Calgary, Calgary, Canada
Email: Afshin.derakhshani@ucalgary.ca

Abstract: Ulcerative colitis is characterized by impaired gut barrier function. Mast cell-derived chymase influences epithelial integrity, but its primary role remains unclear. Using the TAILS technique, we identified chymase/Mcpt4 substrates in colonic epithelial cells, revealing cytoskeletal and junction-associated proteins enrichment. Transepithelial electrical resistance assays confirmed chymase-mediated barrier disruption using T84 cells. Our data indicates that chymase/Mcpt4 plays a role in epithelial dysfunction in ulcerative colitis, suggesting it as a possible therapeutic target.

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) primarily affecting the large intestine (1). In Canada, IBD prevalence was 0.7% in 2018 and is predicted to reach around 1.0% of the population by 2030, posing a significant healthcare burden (2). The adverse effects of existing medications underscore the necessity for novel therapeutic techniques that increase drug efficiency with minimum side effects (3). Although the exact pathogenesis of UC remains unclear, key contributing factors include mucus deficiency, dysbiosis, dysregulated mucosal immunity, elevated inflammatory mediators, tissue damage, and increased epithelial permeability (2, 4). Mast cells (MCs) are commonly acknowledged as primary effectors in type 1 allergic diseases (5). However, they also are involved in various other conditions, including acute and chronic inflammation. (6). This involvement stems from their ability to release stored mediators within their granules. These mediators include cytokines, histamine, serglycin, and proteases such as chymase, tryptase, and carboxypeptidase A (7). Chymase secreted by mast cells can degrade alarmins, including HSP70, Biglycan, and IL-33 (8), as well as proinflammatory cytokines like TNF-α, thereby contributing to the reduction of inflammation (9). At the same time, chymase contributes to intestinal epithelial permeability through a mechanism involving protease-activated receptor (PAR)-2 and matrix metalloproteinase (MMP)-2 (10). Given these opposing effects, its precise role in UC pathology remains unclear. In the current work, the Terminal Amine Isotonic Labeling of Substrates (TAILS) technique and transepithelial electrical resistance (TEER) assay were utilized to investigate how chymase affects epithelial tight junctions and proteins involved in these pathways.

HCT116 cells were treated with recombinant chymase, followed by the TAILS technique to identify specific chymase substrates in colonic epithelial cells. The same method was applied to profile Mcpt4 substrates, a functional homolog of human chymase, in the colons of Mcpt4$^{-/-}$ (n = 6) and wild-type mice (n = 6). Our approach identified significant substrates for Mcpt4 and chymase, revealing enrichment in pathways linked to cytoskeletal organization, tissue integrity, tight and adherens junctions, and cellular responses to stress. Key identified proteins included cadherin-13, desmin, filamin, actin, keratin, collagen type VI, and heat shock proteins, suggesting a crucial role for chymase in maintaining epithelial structure and function.

Furthermore, in vitro TEER assay was performed using T84 cells to assess the functional consequences of chymase activity. Our results demonstrated that chymase, like TNF-α, significantly decreased TEER, indicating compromised barrier integrity and increased epithelial permeability. These findings suggest that chymase might directly disrupt tight and adherens junctions, potentially exacerbating colonic epithelial dysfunction in UC. Further experiments are needed to clarify the precise mechanisms of chymase.

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