doi 10.18699/bgrs2024-7.2-08

Disfunction of intercellular junctions in chronic colitis models

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Key words: chronic colitis; F-actin; tight junctions; adherence junctions; leaky gut

Motivation and Aim: Increased permeability of the intestinal barrier, commonly known as "leaky gut syndrome," is a prevalent functional disorder observed in various gastrointestinal diseases, including inflammatory bowel diseases (IBD), celiac disease and bowel infections. The underlying cause of leaky gut syndrome is the disruption to the structure of tight junctions (TJ) and adherence junctions (AJ) within the intestinal epithelium. Numerous studies focusing on acute IBD suggest that an imbalance between pro- and anti-inflammatory factors leads to impaired distribution of TJ and AJ proteins [1–3]. Researches on F-actin reorganization during inflammation *in vivo* are non-systemic and contradictory, although microfilaments are essential for TJ and AJ formation [4, 5]. We aimed to establish a connection between F-actin stability and intercellular junctions in the context of leaky gut which can develop in various ways. Here, we describe F-actin, TJ and AJ structure and distribution in colons of three murine models of chronic colitis: *Muc2*-KO mice [6], DSS-induced chronic colitis [7] and $CD4^{+}CD45RB^{High}$ cell transfer model [8].

Methods and Algorithms: *Muc2*-KO mice were obtained by the rederivation of previously generated $Muc2^{tm1Avel}/Muc2^{tm1Avel}$ mice on C57BL/6 genetic background in SPF CD1 female mice and backcrossing to C57BL/6JNskrc. For DSS chronic inflammation, WT mice received three cycles of 2 % DSS/water. For cell transfer inflammation, athymic 12-week-old male Nude (Nu/j) mice were injected with sorted $CD4^{+}CD45RB^{High}$ cells obtained from Balb/c mice.

Distribution of AJ and TJ proteins in the descending colon was assessed with immunohistochemistry, using anti-β-catenin (# 610153, BD Transduction Laboratories), anti-Claudin 7 antibodies (# 37-4800, Invitrogen) and Alexa Fluor 568 Phalloidin (# A12380, ThermoFisher Scientific) for F-actin tracking.

To study F-actin interactions, WT mice were administered Latrunculin A (# 100-0562, Stemcell Technologies) or Jasplakinolide (# sc-202191A, Chemcruz) rectally. F-actin polymerization was evaluated by filming fluorescence recovery after photobleaching (FRAP) with CellMask Green Actin dye (# A57243, Invitrogen).

Results: Immunohistochemistry analysis revealed that modulators of F-actin stability Latrunculin A and Jasplakinolide affect not only microfilaments but Claudin 7 and β-catenin localization as well. FRAP timelapses showed decreased polymerization rate of actin filaments in enterocytes of *Muc2*-KO mice. All three models of chronic inflammation demonstrated disrupted structure of actin microfilaments, which anchor the TJ and AJ complexes [5]. Additionally, the localization of the TJ protein Claudin 7 and AJ protein β-catenin on the lateral membranes of enterocytes was also impaired in all three models.

Conclusion: Disruptions in F-actin structure, coupled with altered localization of Claudin 7 and β-catenin, appear to be common features across at least three mouse models of chronic colitis. These features are, potentially, universal characteristics of increased permeability in chronic colitis. Therefore, we suggest that the mechanism of leaky gut syndrome development is as follows: chronic inflammation affects F-actin structure and causes TJ and AJ instability.

Funding: The study is supported by the Russian Science Foundation (RSF) Grant (No. 20-74-10022-П).

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