The role of Akkermansia muciniphila and extracellular vesicles in autophagy pathway regulation

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Abstract

Background and Aim : Akkermansia muciniphila is a next-generation probiotic that digests the mucin in the mucus layer of the intestine. This bacterium plays a vital role in maintaining the gut barrier, regulating metabolic functions and it modulates the immune system, thereby reducing inflammation and promoting a balanced immune response. Research indicates that extracellular vesicles (EVs) derived from A.muciniphila—nano-sized vesicles containing various bioactive macromolecules—can exhibit similar functions to the bacteria itself. Autophagy is a crucial cellular process responsible for maintaining cellular homeostasis by recycling damaged cellular components. This process is key to several physiological processes, including immune responses, cellular stress responses, and the maintenance of the intestinal barrier's integrity. In the context of Inflammatory Bowel Disease (IBD), autophagy has been found to be dysregulated. Critical genes involved in the autophagy pathway, notably ATG5 and ATG16L1, are essential for the formation of autophagosomes, which are vesicles that encapsulate cellular components for degradation. In this study, we seek to examine the expression levels of ATG5 and ATG16L1 in patients with ulcerative colitis (UC) and Crohn's disease (CD) (two types of IBD) compared to healthy controls. We will also investigate the effects of A.muciniphila and its extracellular vesicles on regulating these genes in an inflamed Caco-2 colonic cell line model.

Methods : A total of 20 UC, 20 CD, and 18 healthy gut tissue samples were obtained from patients at the Gastroenterology Clinic of Taleghani Hospital in Tehran, Iran, with written informed consent. RNA was extracted from these tissue samples, and cDNA was synthesized. Real-time PCR was conducted to quantify the expression levels of the genes ATG5 and ATG16L1 in these patients. The Caco-2 colonic cell line was cultured in DMEM media supplemented with 10% FBS. Additionally, A. muciniphila was cultured anaerobically on supplemented BHI agar, and EVs were isolated through ultracentrifugation. To induce inflammation in the Caco-2 cells, interleukin-1 beta (IL-1B) was used. The cells were subsequently treated with A. muciniphila at MOI of 10 and 100, along with its EVs at concentrations of 1 and 10 µg/ml, for a duration of 24 hours. Following these treatments, the expression levels of ATG5 and ATG16L1 were assessed in each treatment group.

Results : Both ATG5 and ATG16L1 were significantly overexpressed in patients with CD and UC compared to control subjects. When comparing the two conditions, ATG5 exhibited a greater upregulation in CD patients, while ATG16L1 showed a more pronounced increase in UC patients. Additionally, ATG5 levels were elevated following inflammation in the Caco-2 cell line, whereas ATG16L1 expression decreased in response to inflammation within this cell model. Treatment with A. muciniphila or an EV at 1 µg/ml significantly reduced the overexpression of ATG5, while treatment with bacteria or an EV at 10 µg/ml markedly increased the expression of the previously reduced ATG16L1 during inflammation.

Conclusion : ATG5 and ATG16L1 are dysregulated in patients with IBD. A. muciniphila and its EVs, which act as a probiotic and postbiotic respectively, could help enhance this dysregulation and restore the balance in the expression of two important genes in the autophagy pathway.

Keywords : Akkermansia muciniphila, Autophagy, Inflammatory bowel disease, Extracellular vesicle, postbiotic, Probiotics