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Ileal pouch of ulcerative colitis and familial adenomatous polyposis patients exhibit impaired autophagy

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Abstract

Total colectomy with ileal pouch-anal anastomosis (IPAA) is the surgery of choice for patients with ulcerative colitis (UC), that are refractory to clinical treatment. Pouchitis is one of the most common complications after this procedure. Previous studies have shown increased pro-inflammatory cytokines in the ileal pouch (IP) mucosa of UC patients. Defects in autophagy have been reported in inflammatory bowel diseases. However, there are no studies on the IP. Therefore, we studied markers for autophagy in the IP mucosa of UC and FAP patients comparing them to controls with a normal distal ileum. We found an impaired macroautophagy mechanism in the IP, which may explain the inflammation predisposition, mainly in the IP mucosa of UC patients.

Key words:

Autophagy, ulcerative colitis, inflammatory bowel disease.

Introduction

Ulcerative colitis (UC) is a chronic intestinal inflammation that can affect the large intestine and rectum. Its etiology is not completely established. Familial adenomatous polyposis (FAP) is an autosomal dominant disease which affects young individuals and is associated with the formation of multiple polyps in the large intestine and rectum, which invariably implies a greater risk of cancer. Both diseases, despite being different, may require the same surgical procedure. The ileal pouch-anal anastomosis (IPAA) is the elective procedure of choice in the surgical management of refractory UC, and FAP with many polyps in the rectum. The main complication after this procedure is the pouch inflammation (pouchitis) that can affect up to 45 percent of patients who are submitted to IPAA for UC, and only five percent of the FAP patients who undergo the same procedure. Defects in autophagy have been reported in inflammatory bowel diseases. However, there are no studies on the IP. Autophagy is an evolutionarily conserved catabolic pathway that consists of selective degradation of cellular components and a homeostatic mechanism that protects cells exposed to stress situations (toxins, starvation). Thus, we evaluated molecules involved in the autophagy pathways in ileal pouch mucosa of UC and FAP patients, even in the absence of clinical, endoscopic and histological inflammation, in order to understand if there is underlying modulation in these pathways that can predispose them to future alterations.

Results and Discussion

Sixteen patients with IP in "J" shape, asymptomatic and with endoscopically normal IP were evaluated. The control group consisted of eight patients with normal colonoscopy. The expression of ULK1, BECN1, ATG16L1, ATG5, MAP1LC3A, BAX, BCL2 transcripts were analyzed by qPCR and Beclin-1, LC3 II, p62 and HSC-70 protein levels by immunoblotting and Inflammatory Bowel Disease Research Laboratory (Faculty of Medical Sciences) and was approved by the Ethical Committee of University of Campinas.

There was a significant decrease in the transcriptional levels of ATG5, MAP1LC3A and BAX in the FAP group ($p < 0.05$). There was also a decrease in the protein level

of Beclin-1 in the UC and FAP groups compared to the control group ($p < 0.05$). Although the LC3II levels by immunoblot were higher in the UC group, total LC3 and LC3/p62 co-localization were lower in the immunofluorescence analysis in the UC and FAP groups compared to the control group ($p < 0.05$). Corroborating these results, there was an increase of p62 by immunoblot in the UC group, compared to controls ($p < 0.05$).

Therefore, we verified decreased of macroautophagy markers in the ileal pouch mucosa of both, UC and FAP, but the mechanisms to explain may be distinct, analyzing data already published. In FAP, decreased autophagy may be related to impaired apoptosis, otherwise in UC, may be mainly due to increased TLR activation. Therefore, autophagy is relevant to the cell survive, since the accumulation of unfolded and abnormal proteins leads to activation of pro-inflammatory pathways. Those evidences of impaired autophagy may explain the prone to inflammation in the ileal pouch mucosa, mainly in UC. This subject deserves further studies and detailed mechanisms, which can help to find out new targets to ameliorate inflammation in the ileal pouch and even in UC.

Conclusions

These findings indicated an impaired macroautophagy mechanism in the IP, which may explain the inflammation predisposition, mainly in the IP mucosa of UC patients.

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