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Title: **Glucose transporter expression in the human colon**

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Monosaccharide transport across the cell membrane is mediated by the expression of glucose transporter isoforms characterized by a tissue-and species-specific distribution, and a different affinity for sugars, polyols, and other carbon compounds (Mueckler et al., 1985). The most widely investigated glucose transporters in the gastrointestinal tract are GLUT2, SGLT1, and GLUT5 (Thorens et al., 1990; Wright et al., 2004; Kellett et al., 2008). Their topographical variations have usually been studied by molecular biology techniques rather than standard immunohistochemical analysis, and most often in the small intestine. The localization and distribution of glucose transporters in the intestinal mucosa of the human colon has not yet been clarified despite evidences that support their role in broader areas of pure glucose absorption and metabolism, such as inflammation, malignancy, and gut microbiota regulation (Uldry et al., 2002; Yu et al., 2005; Garcia-Herrera et al., 2008; Schmitt et al., 2017).

The aim of this study was to investigate by immunostaining at light and confocal microscopy the expression of the major intestinal glucose transporters (GLUT2, SGLT1, GLUT5) in human colorectal mucosa in controls and subjects with inflammatory bowel disease (IBD).

Patients diagnosed with ulcerative colitis (UC, n=18) or Crohn’s disease (CD, n=10) and scheduled for diagnostic colonoscopy were enrolled. Patients who underwent colonoscopy for prevention screening of colorectal cancer or were followed-up after polypectomy or lower gastrointestinal symptoms were designated as the control group (CTRL, n=16). Colorectal samples were obtained from patients undergoing lower endoscopic colonoscopy or recto-sigmoidoscopy. Biopsies of portions of the colonic tract (cecum, ascending colon, transverse, descending, sigmoid colon, rectum) were taken for diagnostic purposes according to the endoscopist’s judgment and for immunohistochemistry (IHC). The biopsies were collected from adjacent sites to compare the level of inflammation in independent samples. A total of 147 biopsies of colonic mucosa were collected for IHC analysis. Inflammatory status of the mucosa at the sampling site was evaluated endoscopically in all biopsies and histologically in 127 out of 147 biopsies by an experienced pathologist who evaluated the mononuclear and polymorphonuclear cell infiltration of the mucosal layer.

For the endoscopic findings, inflammatory status was graded according to the Mayo endoscopic score in the UC patients, and according to the Rutgeerts score in previously resected CD (3 out of 10) patients. For the non-resected CD and CTRL patients, inflammatory mucosal status was graded as documented in the endoscopic report. Based on histological and endoscopic grading, the mucosal status towards inflammation and classification as inflamed or non-inflamed was determined.

Samples were fixed in formaldehyde and processed by embedding in paraffin using standard methods. Sections were cut to 7µm thickness and processed for immunoperoxidase and double immunofluorescence labeling.

The results of IHC analysis of samples from the cecum, ascending and transverse colon were grouped together as data of the proximal tract; the results of samples from the descending, sigmoid colon, and rectum were grouped as data of the distal portion of the large intestine. Percentage of samples showing glucose transporter expression was obtained for inflamed and non-inflamed samples in each patient group.

The study provides evidence that GLUT2, SGLT1, and GLUT5 glucose transporters are expressed in the epithelial cells, mainly located in the brush border membrane, of the mucosa of the large intestine of the IBD and control patients. However, unlike the small intestine, their expression was present only in short epithelial portions, involving a limited number of cells. We observed no important differences in glucose transporter expression between the samples obtained from the proximal and distal tracts and between the different patient groups.

Unexpectedly, GLUT5 expression was also identified in vessels, mainly concentrated in specific areas where the vessels were clustered. Immunostaining with LYVE-1, a specific marker of lymphatic endothelium, and GLUT5 antibodies revealed that GLUT5-immunoreactive (-IR) clusters of vessels were concentrated in areas internal to those that were LYVE-1 positive. GLUT5 and LYVE-1 did not appear to be colocalized but rather showed a close topographical relationship on the endothelium. Based on their LYVE-1 expression, GLUT5-IR vessels were identified as lymphatic. Both inflamed and non-inflamed mucosal colorectal tissue biopsies from the IBD and control patients showed GLUT5-IR clusters of lymphatic vessels.

Our results show the expression pattern of glucose transporters in different portions of the large intestinal mucosa in controls and IBD patients and provide first evidence that GLUT5 expression is associated with lymphatic vessels in controls and IBD patients. This novel finding yields further insight into the characterization of lymphatic vasculature, whose dysfunction is a long-recognized feature in humans with IBD (Geleff et al., 2003; Kaiserling et al., 2003; Fogt et al,. 2004; Alitalo et al., 2005; Pedica et al., 2008; Rahier et al.,

2011; Kerjaschki, 2014). In this regard, GLUT5 expression on endothelial lymphovascular cells may have implications for routine use in the histopathological evaluation of lymphoangiogenesis, also in combination with LYVE-1, a marker of lymphatic endothelium that can be down-modulated under inflammatory conditions.

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