Study of the Colonic Mucus Layer by Mass Spectrometry

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal Björn Folkow, Medicinaregatan 11, Göteborg, torsdagen den 18 december 2014, kl. 9.00

Av

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The thesis is based on the following papers:

I. van der Post, S., Jabbar, K.S., Sjövall, H., Johansson, M. E. V., and Hansson G.C. **The protein composition of the human colonic mucus: reduced levels of core structural components in active ulcerative colitis.** *Manuscript*

II. van der Post, S*., Subramani, D. B*., Bäckström, M., Johansson, M. E. V., Vester-Christensen, M. B., Mandel, U., Bennett, E. P., Clausen, H., Dahlén, G., Sroka, A., Potempa, J., and Hansson, G. C. (2013) **Site-specific *O*-glycosylation on the MUC2 mucin protein inhibits cleavage by the *Porphyromonas gingivalis* secreted cysteine protease (RgpB).** *Journal of Biological Chemistry* **288**, 14636–14646. *Equal contribution

III. van der Post S., Thomsson K. A., and Hansson G. C. **A multiple enzyme approach for the characterization of glycan modifications on the c-terminus of the intestinal MUC2 mucin.** *Journal of Proteome Research in press*

IV. Ambort, D., van der Post, S., Johansson, M. E. V., Mackenzie, J., Thomsson, E., Krengel, U., and Hansson, G. C. (2011) **Function of the CysD domain of the gel-forming MUC2 mucin.** *Biochemical Journal* **436**, 61–70

V. van der Post S., and Hansson G. C. (2014) **Membrane protein profiling of human colon reveals distinct regional differences.** *Molecular & Cellular Proteomics,* **13**, 2277-2287

ABSTRACT

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The mucus covering our internal mucosal surfaces is a part of the innate immune system, and the first line of defense against microbial challenges. The need of an efficient defense system is especially important in the lower parts of the digestive tract where the microbiota reaches its highest density. In the colon, the mucus forms a dense layer that prevents bacteria from accessing the epithelial surface. The gel-forming mucin 2 (MUC2) is the major structural component of the colonic mucus layer, forming large net-like structures by oligomerization in the N- and C-terminal regions. A dysfunctional mucus layer that allows bacteria to pass through and access the underlying epithelium has been associated with inflammatory bowl diseases such as ulcerative colitis. However, detailed understanding of the molecular mechanisms behind the defective mucus layer is lacking. This lack of knowledge can largely be explained by the limited information regarding the composition and processing of the mucus during normal conditions. This thesis aims to broaden the knowledge regarding the protein composition of the human colonic mucus, and the molecular properties of the heavily glycosylated MUC2 mucin.

Proteomic and mass spectrometry approaches were used to characterize the composition of the human colonic mucus layer in health an disease, and to determine how alterations in protein abundance and modification of the MUC2 mucin affect the function of the mucus gel. Our results showed that the human colonic mucus is comprised of approximately 50 proteins. The protein composition of the mucus layer was shown to be unaffected in patients with ulcerative colitis, though the relative abundance of 13 mucus proteins including the structural components MUC2 and FCGBP were shown to be decreased during active disease.

The mucin protein family is characterized by a heavily *O*-glycosylated core that is resistant against proteolytic degradation. However, our results showed that the C-terminal part of the protein is also modified by *N*- and *O*-glycans, and that site specific *O*-glycosylation plays an important role in protecting the protein from proteolytic degradation by bacterial proteases. In addition, we could correlate the relative abundance of various glycosyltransferases required for *O*-glycosylation in the different parts of the colon, to the previously characterized segmental pattern of terminating glycans on the MUC2.

Taken together, the results from this thesis show that the human colonic mucus is composed of a relatively small number of proteins that are organized around the heavily *O*-glycosylated MUC2 mucin, and suggests that decreased amounts of the core mucus proteins in combination with impaired *O*-glycosylation of the MUC2 renders the mucus layer more permeable to bacteria and susceptible to proteolytic degradation.

Key words: MUC2, mucin, intestine, proteomics, mass spectrometry
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